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THE EFFECTS OF HIGH TEMPERATURE ON BRISTLE FREQUENCIES IN SCUTE AND WILD-TYPE MALES OF DROSOPHILA MELANOGASTER

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Received December 1, 1938

INTRODUCTION

DURING the past twenty-five years there has appeared a considerable amount of literature concerned with temperature-effective period studies on mutant races of *Drosophila melanogaster*. Adult characters which have drawn the most attention have been the compound eye, the wing, and the bristles. Most of the work on the eye has been done with Bar and its alleles (see especially DRIVER 1926 and 1931, LUCE 1931, and MARGOLIS 1935). Wing studies have been confined largely to vestigial and its alleles (see especially RIEDEL 1934, STANLEY 1935, HARNLY 1936, HARNLY and HARNLY 1936, and LI and TSUI 1936). The bristle studies have been on the bristle-removing genes, Dichaete (PLUNKETT 1926) and scute (CHILD 1935 and 1936, IVES 1935). It has been found to be general in most of these studies that under the conditions of the experiments the adult expression of these several mutants is affected by a change in developmental temperature over the viable temperature range (14° to 31°C) during only a limited developmental period. This period has come to be known as the temperature-effective period (TEP) of the mutant character.

This paper reports a series of experiments on scute (*sc*) using brief exposures to lethal temperatures (36° and 40°C). A preliminary account of part of this work has already been published (IVES 1935).

EXPERIMENTAL METHODS

The methods used in these experiments were described in the preliminary account. It has since been found, however, that the variation in experimental conditions (food and temperature) was enough to change bristle frequencies in the controls so that more than rough comparisons of data collected several months apart are unreliable. This accounted for many of the irregularities of the earlier report. Accordingly, all comparisons have since been made between sib control and experimental flies which were raised under as similar conditions as possible. Special attention was given to the number of larvae growing together in a culture, to the age of the parents, and to temperature and food conditions.

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Flies in part of the experiments were raised on moldex-treated food, (BRIDGES 1937). The moldex affected some of the bristle frequencies, possibly by reducing the supply of yeast. It did not appear to affect the temperature responses of the bristles.

PLAN OF THE EXPERIMENTS

Experiments were carried out with *sc* males of two types: from *sc* females against *sc* males, and from *sc* females against vermilion (*v*) carnation (*car*) males; and with males of a wild type stock derived from the above *sc*-by-*v car* cross. The *sc* and *v car* stocks were inbred previous to beginning the experiments. Left-right correlations (PLUNKETT 1926) of the bristles considered were throughout the experiments negligibly small (less than 0.1) in both types of *sc* males in the control lines.

Experiments were carried out with both of the above *sc* matings at 40°C and at 36°C; and with the wild-type flies at 36°C, exclusive in each case of the first hour (the "a" exposure) during which the food temperature was rising from the control temperature of 25°C. The longest exposures were generally just under the limit of viable exposure for the majority of the culture population in each test. Variation in food temperature from culture to culture was mostly between 40 and 41°C, and 36 and 37°, respectively. The control food temperature, though constant to within about 0.2° for each section of an experiment, varied between 25 and 26° from section to section, with most sections between 25 and 25.5°. Controls were raised for each section individually.

The bristles considered in these experiments were as follows: anterior and posterior scutellars (AS and PS), anterior notopleurals (AN), postverticals (PV), ocellars (OC), and anterior and medial orbitals (OR). As reported by CHILD (1935), it was found that the last of these bristles could not be distinguished as to anterior or medial position in *sc* males and that they were practically never present together on the same side of the fly. (They were observed so together but once in some 500,000 half-fly observations connected with these experiments.) They have therefore been grouped as OR in these experiments as they were in those of DR. CHILD.

Bristle frequencies have been computed on the basis of the half-fly unit (PLUNKETT 1926). A difference between two frequencies has been considered significant when it equals or exceeds twice its standard error. In the data to be discussed, most of the differences between control and experimental frequencies are as large as four times their standard errors. To obtain low standard errors generally not less than 1000 half-fly observations were made for each length of exposure tested. The controls were generally considerably larger in number.

The experiments were designed to test the effects of graded lengths of

exposures to high temperatures during a number of developmental periods covering most of the development preceding the visible formation of bristles (ROBERTSON 1936). Thus, in the 40° experiments, exposures ranged from half an hour to three hours in length and tested a part of each of the egg, three larval, and pupal stages of development. In the 36° experiments two lengths of exposures were used, and each of the larval instars and the early pupal period were tested. The developmental period in each case has been calculated in hours from the beginning of the egg-laying period. Its spread represents the sum of the egg-laying and exposure periods. It is known that there was variation in developmental time for the larvae of a culture population, making the calculated time only approximate. It probably represents the position of the considerable majority of the larvae, since late-hatching flies were not classified for bristles.

No attempt was made to determine exactly the relation of the tested developmental periods to such physiological landmarks in development as the hatching of the egg, the larval molts, and pupation. But it was noted that pupation (cessation of larval movement and eversion of anterior spiracles) took place in the control series at an approximate mean of 120 hours after egg laying. From a number of studies on the time interrelations of the above stages of development (see POWSNER 1935 for one) the following general time chart is suggested for practical use here:

Egg stage	0 to 24 hours
First larval instar	24 to 48 hours
Second larval instar	48 to 72 hours
Third larval instar	72 to 120 hours
Pupal stage	120 hours on.

A comparison of this chart with the tables of data to be presented will indicate the developmental stage during which the indicated treatment was given.

THE EXPERIMENTAL DATA

The 40°C experiments

The data for the *sc* males in the 40° experiment with the *sc*-by-*v car* mating are summarized in table 1 as frequencies of bristles present per 100 half-flies. Where no effect of the temperature treatment was apparent following any of the exposures, all of the exposure series, from "a" through three additional hours of 40°C, have been added together. When a temperature effect was apparent, the data from the shortest exposure to show the effect and the data from all longer exposures have been added together. Thus each frequency represents a mean of several groups of data. The shortest exposure included in each frequency is indicated in each case

above the frequency. As a general rule, increasing the length of the exposure did not increase a temperature effect, once the latter had been established. Left-right correlations were similar to their respective controls, less than 0.1, and not statistically different from 0, indicating that the effect was upon the population as a whole. With each experimental frequency is given the corresponding control frequency and the standard

TABLE 1

sc ♀♀ × *v car* ♂♂—*40°C.—Bristles present per 100 half-males.*

GROUP	PS	AS	AN	PV	OC	OR
0 to 10 hours	a	a	a	a	a	a
(a—2 hours)	0.08	2.39	0.82	0.58	11.28	12.84
Control	0.29	2.60	0.22	0.43	3.39	7.13
S.E. of diff.	0.17	0.64	0.29	0.29	1.03	1.18
20 to 30 hours	a	a	a	1½ hrs	½ hr.	½ hr.
(a—3 hours)	0.36	2.49	0.71	0.22	8.53	7.50
Control	0.52	2.39	0.57	0.62	4.53	4.87
S.E. of diff.	0.09	0.20	0.11	0.11	0.39	0.38
48 to 58 hours	a	a	a	½ hr.	½ hr.	a
(a—3 hours)	0.24	1.44	0.39	0.09	9.23	8.83
Control	0.39	2.23	0.43	0.30	3.52	5.55
S.E. of diff.	0.11	0.27	0.13	0.09	0.42	0.47
72 to 82 hours	a	a	a	a	a	1 hr.
(a—3 hours)	0.26	2.24	0.78	0.08	9.95	4.95
Control	0.11	2.12	0.11	0.22	5.37	6.88
S.E. of diff.	0.10	0.38	0.13	0.11	0.63	0.67
96 to 106 hours	a	a	a	a	½ hr.	a
(a—3 hours)	0.25	2.03	0.53	0.30	5.70	4.68
Control	0.36	2.26	0.40	0.31	3.50	5.81
S.E. of diff.	0.12	0.29	0.13	0.11	0.40	0.45
120 to 130 hours	a	a	a	a	a	1 hr.
(a—3 hours)	0.13	1.97	0.34	0.61	6.81	5.20
Control	0.29	2.60	0.22	0.43	3.39	7.13
S.E. of diff.	0.15	0.47	0.15	0.21	0.61	0.78

error of the difference between the two frequencies (S. E. of diff.). Under the heading "Group" are included the developmental period during which treatment was given, and in parentheses under that, the minimal and maximal exposures used. In the 0 to 10 hour and 120 to 130 hour groups only even hour exposures were used; but in the others the length of exposure time was increased by half-hour steps.

The data of table 1 show clear effects of the temperature treatment on the OC and OR frequencies in each of the several developmental periods

tested. The OC frequency was increased in each period. The OR frequency was increased in the egg and first two instar periods and was lessened in the two-third instar periods and in the early pupal period. The frequencies of the other bristles are so small in the control data that small changes in them in either direction are not easily detectable, especially when the frequencies are interpreted in relation to a threshold amount of bristle-forming substance (CHILD 1936). It is apparent, however, that large changes (such as will be noted in the 36° experiment in the cases of the PS and AS frequencies) were not brought about by the 40° treatment. The apparent small changes in frequencies of PS and AS bristles are probably not significant, for when the six exposure groups comprising each frequency are considered individually their differences from their controls in each instance are not consistent in direction. The experimental frequency varies above and below the control, apparently at random. This is not true in the case of the PV frequency which, beginning with the one and one half hour exposure in the 20 to 30 hour group and with the half hour exposure in the 48 to 58 hour group, was consistently below the control frequency in each of the succeeding higher exposures. It appears likely, therefore, that this represents a real effect of the temperature. The AN frequency was consistently different from the control in only the 0 to 10 and 72 to 82 hour groups. In each case the control frequency was lower than usual, making the temperature effect a doubtful one.

Correlation coefficients were calculated for the one-by-one combinations of frequencies represented in table 1. None of them were significantly different from zero. While the correlation coefficient does not give an accurate measure of the intensity of association of bristles with each other, the absence of correlation here probably indicates the same sort of independence of one bristle from another as was found by CHILD.

Following the *sc*-by-*v car* experiment, a similar but less extensive series of tests was run with the *sc*-by-*sc* mating. There appeared to be consistent differences in some of the control frequencies in the two types of *sc* males. The changes from the *sc*-by-*v car* frequencies in bristles present per 100 half-males were in the order of from 0.6 to 0.06 for the PS bristles, from 3.3 to 0.6 for the AS, and from 5.2 to 12.1 for the OR, when several representative control series of different population densities were averaged together for each mating. There did not appear to be significant differences in the other frequencies. The differences noted here maintained themselves later in a test of the two matings raised together in time and under similar environmental conditions.

The data from the *sc*-by-*sc* 40° experiment are summarized in table 2 in a form similar to that of table 1. Exposures of less than one hour beyond a were not used. The exposures included in each section of the data are

indicated under the statement of the developmental period tested. One hour exposures were not tested in the 48 to 58 and 72 to 82 hour groups. In the 96 to 106 and 120 to 130 hour groups each of the tested exposures was lethal to many larvae and pupae, making necessary the summation of the three exposures in each case to secure significant numbers of observations.

TABLE 2

sc ♀♀ × *sc̀* ♂♂—40°C.—*Bristles present per 100 half-males.*

GROUP	PS	AS	AN	PV	OC	OR
0 to 10 hours (1 hour)	0.14	0.97	2.50	1.53	4.73	13.48
Control	0.07	1.11	0.81	0.88	2.50	9.93
S.E. of diff.	0.16	0.46	0.63	0.52	0.90	1.51
20 to 30 hours (1; 2; 3 hours)	0	0.73	1.45	1.00	8.04	20.92
Control	0	0.37	0.57	0.63	3.86	16.97
S.E. of diff.		0.25	0.33	0.30	0.78	1.28
48 to 58 hours (2; 3 hours)	0	0.42	1.00	0.25	6.28	14.98
Control	0	0.11	0.82	0.71	4.24	13.52
S.E. of diff.		0.20	0.33	0.20	0.78	1.16
72 to 82 hours (2; 3 hours)	0.30	1.59	4.78	1.33	5.74	11.89
Control	0.13	1.05	0.98	1.16	2.78	7.47
S.E. of Diff.	0.11	0.27	0.43	0.25	0.49	0.69
96 to 106 hours (1; 2; 3 hours)	0.27	2.22	3.91	1.16	9.68	6.04
Control	0.27	1.64	1.54	0.51	5.63	5.87
S.E. of diff.	0.18	0.50	0.63	0.35	0.98	0.83
120 to 130 hours (1; 2; 3 hours)	0	0.83	1.28	0.60	4.53	5.14
Control	0.04	0.56	0.91	0.37	5.03	5.06
S.E. of diff.	0.02	0.27	0.33	0.22	0.63	0.66

As in the *sc*-by-*v car* experiment, the clearest effects of the temperature in the data of table 2 are in the OC and OR frequencies. The OC frequency was increased in all but the early pupal period. The OR frequency was increased through the early third instar period; but it was unaffected in the later third instar and early pupal periods. The AN frequency was also affected in this mating, being increased in the egg, first instar and third instar periods. The PV frequency was affected, if at all, during only the second instar, when it appears to have been lessened. As in the *v car*

mating, the PS and AS frequencies did not appear to be significantly affected at any time.

A comparison of the data of tables 1 and 2 indicates small but probably significant differences in response to temperature treatment with respect to the AN, PV, OC, and OR frequencies—mainly in duration of the 40° TEP. The PV, OC, and OR TEP's are longer in the *v car* mating; the AN TEP is longer in the *sc*-by-*sc* mating. Differences will be shown to exist also in the responses of the two types of *sc* males to 36°C.

The 36°C experiments

Preliminary tests at 36° with the *sc*-by-*v car* mating showed that exposures as brief as four hours during the first and second instars produced no significant changes in bristle frequencies. Subsequent experiments were made with much longer exposures. The results of these tests are summarized in table 3. The most significant frequency changes are those of the PS and AS bristles. These frequencies were increased during each of the larval periods tested, although the affect on the PS frequency is on the border line of significance in the second instar. The AN frequency was not affected at any time. The PV frequency was lessened during every period tested. The OC frequency was very irregularly affected. It was unchanged in the first instar and in the first half of the third instar. In the second instar it was very strongly increased. During the late third instar period it appears to have been lessened by the 24 hour exposure and increased by the 30 hour exposure in each case by three times the Standard Error of the difference. In the early pupal period it was again considerably lessened. The OR frequency was increased during every period, the increase in the late third instar series appearing only in the 30 hour group.

It is evident in the data of table 3 that the effects on the PS and AS frequencies were most pronounced during the third instar series; and that the 30 hour exposures produced a greater change than did the 24 hour exposures. The first of these observations suggests a period of greater sensitivity, perhaps related to CHILD'S viable temperature TEP. Left-right correlation coefficients of these bristles in these series were comparatively high, being between 0.2 and 0.5 as compared to control coefficients of less than 0.1. These coefficients are high enough to indicate that the temperature effect was not uniform in all the larvae so far as the PS and AS bristles were concerned. They suggest, too, that the higher frequencies of the longer exposure groups are the result of more larvae having been affected by the temperature with respect to these bristles.

When the data are compared with those of table 1, the 40° data from the same mating, it is clear that there is a fundamental difference in the affects of nearly lethal exposures to the two temperatures on some if not all of

the bristles considered. The differences are greatest in the responses of the PS, AS, and OC bristles. The differences between the controls in these two series are due partly to the inclusion of moldex in the culture media and

TABLE 3

sc ♀ ♀ × v car ♂ ♂—36°C.—Bristles present per 100 half-males.

GROUP	PS	AS	AN	PV	OC	OR
24 to 53 hours (16; 22 hours)	2.43	14.00	1.93	1.07	9.86	6.78
Control A	0.76	4.26	1.60	1.98	10.58	3.27
S.E. of diff.	0.47	1.08	0.51	0.48	1.17	0.83
44 to 74 hours (24 hours)	1.67	8.22	1.11	0.43	12.90	4.02
Control B	1.13	5.38	1.72	1.51	6.52	1.78
S.E. of diff.	0.41	0.86	0.40	0.32	1.01	0.57
45 to 82 hours (30 hours)	1.49	10.54	1.88	0.63	22.50	6.17
S.E. of diff. from control A	0.41	1.03	0.53	0.45	1.45	0.83
68 to 101 hours (24 hours)	7.26	12.88	2.58	0.53	10.22	6.36
Control C	0.90	5.64	2.42	1.64	10.61	2.69
S.E. of diff.	0.73	1.02	0.52	0.30	0.99	0.73
68 to 105 hours (30 hours)	16.39	22.25	2.26	0.68	11.48	4.83
S.E. of diff. from control A	1.03	1.27	0.54	0.45	1.22	0.76
92 to 126 hours (24 hours)	12.35	15.50	3.46	0.47	7.49	2.81
Control D	0.99	6.57	2.97	1.41	10.63	2.29
S.E. of diff.	0.83	1.05	0.58	0.32	0.95	0.52
90 to 128 hours (30 hours)	20.44	22.75	2.18	0.27	14.84	8.31
S.E. of diff. from control C	1.51	1.60	0.60	0.29	1.41	1.06
116 to 148 hours (25 hours)	2.04	4.69	1.80	0	6.25	6.62
S.E. of diff. from control C	0.52	0.81	0.53	0.22	1.00	0.91

partly to a control temperature of 26° during the most of the 36° experiment. Preliminary experiments at 35 to 37° with non-moldex food and at a control temperature of 25° indicated the same sort of temperature re-

sponses as in table 3. The differences between the 40° and 36° bristle responses are due therefore to differences in the nature of the effects of the two temperatures and not to differences in other experimental conditions.

TABLE 4

sc ♀♀ × *sc* ♂♂—*36°C.—Bristles present per 100 half-males.*

GROUP	PS	AS	AN	PV	OC	OR
15 to 34 hours (6; 12 hours)	0.04	0.67	1.55	0.84	6.87	16.66
Control	0.09	0.41	0.63	0.68	5.47	13.25
S.E. of diff.	0.07	0.22	0.30	0.26	0.71	1.05
24 to 57 hours (12; 24 hours)	0.08	1.23	1.70	0.39	5.01	16.96
Control	0.06	0.17	0.61	0.61	3.09	8.34
S.E. of diff.	0.10	0.32	0.40	0.25	0.73	1.23
48 to 80 hours (12; 24 hours)	0	0.35	1.48	0.21	4.43	7.94
Control	0	0.07	0.70	0.42	2.23	8.93
S.E. of diff.		0.18	0.39	0.21	0.67	1.04
72 to 92 hours (12 hours)	1.23	5.82	0.49	0.49	8.28	6.56
Control	0	0.59	1.61	0.46	8.38	15.83
S.E. of diff.	0.32	0.70	0.46	0.29	1.23	1.43
72 to 107 hours (24 hours)	7.82	16.84	0.60	0.34	4.90	5.50
Control	0	0.59	2.10	0.45	4.06	8.42
S.E. of diff.	0.79	1.05	0.60	0.31	0.99	1.27
96 to 116 hours (12 hours)	0.22	3.99	3.99	0.11	3.66	4.88
Control	0.15	0.26	0.70	0.50	2.92	8.08
S.E. of diff.	0.17	0.66	0.67	0.16	0.69	0.86
120 to 140 hours (12 hours)	0.15	0.30	0.89	0.30	3.26	6.07
Control	0.15	0.26	0.70	0.50	2.92	8.08
S.E. of diff.	0.16	0.23	0.39	0.24	0.74	1.03

Following the above 36° experiment a similar experiment was carried out with the *sc*-by-*sc* mating, the data from which are summarized in table 4. This mating could not stand 30 hours at 36° at any period of development. Exposures as brief as three hours proved fatal in early embryonic development; and 24 hours was intolerable during the 96 to 116 and 120 to 140 hour periods. The data show that the only significant effects on the PS frequency occurred during the first half of the third instar.

The AS frequency was increased during the second and both third instar periods. The frequencies of these bristles in the controls, however, was so low that lesser increases such as indicated in the *v car* cross in table 3 would probably not have been detected. As in that mating, the temperature effect here was greatest for these bristles during the third instar and during its longer exposure. The AN frequency was increased during the first and second instars, and during the late third instar. It was lessened during the first half of the third instar. The PV frequency was lessened during the late third instar, and apparently unaffected at other times. The OC frequency was increased during the first and second instars, and unaffected in later periods. The OR frequency was increased during the first instar; it was not significantly affected during the second instar; and it was lessened in the third instar and early pupal periods.

A comparison of these data with those of table 3 indicates differences in the responses of the two types of *sc* males to 36° which are clear in the cases of the AN, PV, OC, and OR bristles, and which may also extend to the PS and AS bristles. Considered together with the similar comparisons of the data of tables 1 and 2 and of their respective controls, which were made above, they suggest strongly the presence of genetic modifiers of *sc* which differ in the *sc* and *v car* stocks and which determine to some extent the type of temperature response observed.

A comparison of the data of tables 4 and 2 indicates, as did the similar comparison between tables 1 and 3, a difference in the responses of genetically similar *sc* males to 36° and 40°. The differences in this case, too, extend to some extent to every bristle considered.

It is worthy of note here that the differences in the effects of 36° and 40° on either type of *sc* male are greater than are the differences in response of the two types of *sc* males to either temperature. For example, the data of tables 4 and 2 resemble each other much less than do the data of tables 4 and 3, with respect to the temperature effects indicated. Thus, in these experiments the temperature treatment given appears to be more important than the genetic modifiers present in determining the resultant bristle-frequency response. Whether this would be true in experiments involving *sc* males with other genetic modifiers can not be stated. Even here the statement is not equally applicable to all the bristles considered. The AN bristle response in table 4 resembles much more that in table 2 than it does that in table 3, considering the several developmental periods as a group. This indicates that the modifiers are of more importance for the AN bristle in these experiments. For the other bristles, however, the temperature treatment appears to be the stronger determining factor in the nature of the response.

After these experiments with *sc* males a 36° experiment was carried out

with a wild-type stock which contained the normal allele of *sc* from the *v car* stock and the normal alleles of *v* and *car* from the *sc* stock. The autosomes were in non-regulated proportions from the two stocks. The cytoplasm was from the *sc* stock. The data from the males of this experiment are summarized in table 5. Each bristle frequency per half-male was so

TABLE 5

Wild-type ♂♂—36°C.—Bristles present per 100 half-flies.

GROUP	PS	AS	AN	PV	OC	OR
Control	98.90	99.54	100	100	99.90	99.97
24 to 55 hours (12; 34 hours)	96.17	98.62	100	100	100	100
S.E. of diff.	0.51	0.31				
48 to 81 hours (12; 24 hours)	97.06	97.58	100	99.92	100	100
S.E. of diff.	0.38	0.33		0.06		
72 to 94 hours (12 hours)	86.20	93.86	100	100	100	99.87
S.E. of diff.	1.26	0.88				0.13
72 to 107 hours (24 hours)	49.44	61.60	100	97.62	99.31	98.71
S.E. of diff.	1.59	1.54		0.48	0.26	0.36
96 to 124 hours (12 hours)	95.94	95.18	100	99.62	99.77	99.32
S.E. of diff.	0.57	0.60		0.17	0.14	0.23
120 to 154 hours (12 hours)	98.61	98.93	100	99.89	100	99.89
S.E. of diff.	0.42	0.36		0.11		0.11

high that an increase in frequency in the treated lines would not have been apparent. A decrease in frequency was detectable, however. It can be seen from the table that there was a significant decrease in the PS and AS frequencies in each of the larval period series, with a relatively very large decrease in the third instar series. For practical considerations this effect is the reciprocal of the effect on *sc* males of comparable genetic background shown in table 3. With respect to the other bristles, the only apparent effect of the treatment occurred in the third instar series, in the period of greatest effect on the PS and AS frequencies. With the exception of the AN frequency, which was unaffected in either experiment, the other bristle frequencies do not appear to bear any respective relation to each other in their temperature responses in tables 3 and 5.

DISCUSSION

The data of tables 1 through 5 indicate the kinds of changes produced in bristle frequencies in Drosophila males by nearly maximum exposures to temperatures above the viable range. The data of the first four of these tables indicate that such changes may be produced in *sc* males for one bristle or another during practically any period of development previous to visible formation of the bristles in the young pupa. The data of table 5 indicate that some of the bristle frequencies of wild-type flies are also affected by such treatment, some of them in a direction opposite to the effect in *sc* males.

Comparisons of the data of tables 1 through 4 with each other have been discussed above. They make clear the following observational conclusions. The nature of the temperature response in bristle frequencies in *sc* males depends upon (1) the temperature treatment given, (2) the developmental period during which the treatment is given, (3) the modifiers present in the treated stock, and (4) the bristles which are considered.

Since we do not know the chemistry of bristle formation these data can not be discussed with reference to specific substances involved. They must therefore be discussed in terms of a general and theoretical developmental scheme. A number of such schemes have been suggested.

The non-existence of a pattern relationship of the bristles in *sc* such as developed by DUBININ (1929) and others, and by STURTEVANT and SCHULTZ (1931) was pointed out by CHILD (1935). His results have been confirmed by calculations from the data in both control and treated lines in some of these experiments. Similarly confirmed is CHILD's evidence of the non-existence of such a physiological gradient scheme as proposed by GOLDSCHMIDT (1931). The bristles of the *sc* pattern appear to develop independently of each other in *sc* flies.

A very general theory, currently known as the "rate theory," was proposed by PLUNKETT (1926) and has since been developed and applied by him (1932) and others in interpreting the effects of both genes and environment upon the adult characters of an organism. (See POWSNER 1935 for diagrammatic illustration.) Based upon orthodox concepts of physical chemistry and the general concepts of genetics, this theory postulates that both genes and environmental forces (such as temperature) affect a given phenotype (such as that of *sc*) by changing the rate or duration, or both, of processes—physical, chemical, biological—involved in the development of the phenotype. The effects on duration, which can be, to some extent, directly measured, are interpreted as being determined in turn, by effects on rates of other developmental processes.

The data of the above tables can be explained on such a broad and generalized theory; but the number of arbitrary assumptions which must

be made for a detailed analysis is too great to make such an analysis profitable at this time. Using the response of the OC bristle in the 40° experiments and the responses of the PS and AS bristles in the 36° experiments of both *sc* and wild-type males, the author, for his own satisfaction, has worked out most of the details for one such schematic analysis in each case, with the aid of DR. CHILD. The above data, while not useful at present as confirmatory evidence for the rate theory, are not in conflict with it.

A comparison may be made between the TEP's found in these studies and the TEP found in CHILD'S experiments. The difference, as already noted, is very marked, since in this case the TEP varies distinctly from bristle to bristle and occupies every part of bristle development for one or another of the bristles. In CHILD'S experiments the TEP was approximately the same for all bristles at all temperatures studied, being limited to the latter half of the third instar when a culture population was considered. The simplest explanation of this apparently fundamental difference between the two groups of data is that many more of the reactions involved in bristle formation are susceptible of effective change by high temperature treatments than by viable temperature treatments. In fact, the differences in the data from the two types of treatment—and especially the considerably more complex nature of the data from high temperature treatments—may be interpreted as indicating the presence of more developmental reactions concerned with bristle formation than one is led to suspect from the data of the viable temperature studies alone. CHILD'S rather strictly limited TEP led him to conclude that the effect of temperature was in the main on the same reaction at each bristle site, and that small differences between bristle locations with respect to rate or duration of the temperature-affected reaction satisfactorily account for the observed differences in amount and direction of change in the bristle frequencies. It can not be shown that the same reasoning does not apply to the results of these high temperature studies. But the data picture is so much more complex—marked differences in TEP position and length, both between bristles and between the 36° and 40°C experiments—that such an explanation seems too simple in this case. The results suggest rather that we are probably concerned here with other reactions which are not detectably affected by changes within the viable temperature range. We may carry this analysis a step further and suggest that the differences in bristle frequency responses to 36°C as compared to the respective responses to 40°C indicate that the reactions detected by the two treatments are for most of the bristles different within themselves. Thus we may consider each of the three treatments—viable temperature, 36°C, and 40°C—as being specific for certain reactions which respond detectably to it and not detectably, if at all, to either of the other two.

GOLDSCHMIDT (1938) has intimated that such a comparison of viable and lethal temperature data is not permissible because the high temperature treatment is probably "destructive." This is a generally accepted interpretation of high temperature effects on living matter and many substances associated with it. However, biochemical studies on such substances as enzymes generally reveal "destructive" or "inactivating" temperatures to be considerably above those used in this series of experiments. And in the particular case in point this criticism does not carry so much weight as it may at first seem to. The variations in bristle frequencies which are brought about by the two types of treatment, with viable temperatures and with lethal temperatures, are not markedly different in magnitude, considering them as a whole. And while the effect of high temperature, especially of 40°C, seems to cause a reaction to go to completion within one or two hours, or even less (no increase in effect with increase in length of exposure), the temperature-effective period for the individual fly through the viable temperature range is itself not a long time. It may be that in both cases we are dealing with reactions which take place quickly and that there is no difference in the strictly temperature effect. On the whole, the differences between the data from the two types of treatment are not such as to suggest at present that in one case the effect of temperature is preponderantly "destructive" and in the other "constructive," in whatever sense one chooses to use those terms with reference to the bristles considered. It seems better, for the present at least, to interpret the differences between the viable and lethal temperature data as has been done in the preceding paragraphs.

Studies upon mutant character TEP's appear to have been made originally with the hope of detecting periods of specific gene activity. It is clear, however, that such a direct relation between the two can not be determined by methods thus far reported. The effects of the temperature treatment may be upon reactions whose components or products are later used in reactions controlled by a given gene or its products; or they may be directly upon such gene controlled reactions; or they may be upon subsequent reactions involving the products of such gene controlled reactions. The TEP is only the time in development during which such an effect is produced; it does not indicate which of the above types of effect is being produced. It is the time when the phenotype is changed by the experimental technique used, the relation of the change to specific gene action remaining obscure. This is as true for CHILD'S TEP as it is for the TEP's detected by higher temperatures. It is also true for all TEP studies thus far reported.

It may be argued that the temperature effects on *sc*-affected bristles are not on the effects of the *sc* gene (in any of the three ways outlined

above); but that instead they are upon the effects of other bristle-frequency-modifying genes which the presence of the extreme modifier, *sc*, makes visible. When the bristle frequency is interpreted on the basis of CHILD'S (1936) application of the threshold principle this argument is strengthened. In the absence of *sc* such modifiers might well be unable to move the amount of bristle-forming substance detectably nearer to the threshold amount necessary for bristle formation, when a population of flies is considered. A comparison of the data of table 3 with those of table 5 sheds some light on this point. In the one case the data are from *sc* flies, in the other from non-*sc* flies. From the comparison of *sc* males of the *sc*-by-*v car* mating with those of the *sc*-by-*sc* mating evidence was obtained for the presence of genetic modifiers of *sc*. PAYNE (1920) found two such modifiers. Doubtless there are others. In addition, we know that there are many mutant genes which affect bristle frequencies. Some of these are known to respond to viable temperature changes in their bristle effects. It is probable that their normal alleles do also exert some influence on bristle frequencies. We may consider, then, that for practical purposes the differences between the temperature responses of *sc* males in table 3 and the closely related wild-type males in table 5 are due to the effects of temperature on *sc* plus the group of modifiers in table 3 and on the modifiers alone in table 5. The wild-type allele of *sc* is here considered only as one of a large group of modifiers and not necessarily any more important in its temperature response than any of the other wild-type alleles of known bristle-affecting mutant genes. From the differences between the two tables it is clear not only that there is a marked effect of temperature on the ultimate effect both of the *sc* gene itself and of the modifiers as a group but also that the response of *sc* is opposite in direction to the response of the modifiers as a group, at least for the bristles most obviously affected. The strength of this statement is lessened perhaps by the fact that we can not determine, from these data at least, how much of this difference is due to possible qualitative effect of *sc* on the temperature response of the modifiers, individually or as a group.

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SUMMARY

(1) Bristle frequencies were tabulated for two races of *sc* males treated with nearly maximum exposures to 40°C and to 36°C, and for closely related wild-type males treated with 36°C, the treatments varying in length and being given at various times in egg-larval-pupal development.

(2) In the 40° experiments the OC frequency was increased during nearly all of bristle development; the AN, PV, and OR frequencies were less extensively affected; and the PS and AS frequencies were unaffected.

(3) In the 36° *sc* experiment the scutellar frequencies were increased during the larval period and especially in the third instar. The other bristles were less extensively affected.

(4) In both 36° and 40° *sc* experiments there were consistent differences in both control bristle frequencies and in frequency responses to the temperature treatment between males from a *sc*-by-*v car* cross and those from a *sc*-by-*sc* cross.

(5) In the wild-type 36° experiment the scutellar frequencies were lessened in each tested larval period and especially in the third instar. The other bristle frequencies were only slightly affected.

(6) The effects of 40° and probably of 36° on bristle frequencies do not appear to be increased by lengthening the exposure period beyond that needed to establish the effect in the entire population.

(7) The nature of the effects of temperature on the bristle frequencies of *sc* males depends upon the temperature used, the developmental period treated, the genetic modifiers present, and the bristles considered.

(8) The absence of correlation between bristles in both control and treated lines confirms the results of CHILD in demonstrating the nonexistence of "pattern" relationship or "gradient systems" in *sc*.

(9) The data are explainable in terms of the "rate theory" of gene and environmental effects on developmental processes, but are not critical for the theory itself.

(10) Extensive differences between the results following the 40°, 36°, and CHILD's viable temperature treatments are interpreted as indicating that probably different reactions are affected detectably by each of the three treatments.

(11) The data do not indicate that the effects of high temperature are necessarily different in nature from those of viable temperature.

(12) The TEP is the time when the phenotype is changed, but it does not indicate the relation of the change to specific (mutant) gene action.

(13) The differences between the 36° effects on *sc* and wild-type males are interpreted as indicating that the temperature affects the ultimate bristle effect of both *sc* and the group of modifying genes, and that in this

case the direction of the effect on *sc* is opposite to that on the other bristle modifiers as a group with respect to the bristles most clearly affected.

LITERATURE CITED

BRIDGES, C. B., 1937 Revised data on culture media and mutant loci of *Drosophila melanogaster*. Tab. Biol. **14**: 343–353.

CHILD, G. P., 1935 Phenogenetic studies on *sc-1* of *Drosophila melanogaster*. I. The association between bristles and the effects of genetic modifiers and temperature. Genetics **20**: 109–126.

1935 II. The temperature-effective period. Genetics **20**: 127–155.

1936. III. The effect of temperature on *sc-5*. Genetics **21**: 806–816.

DRIVER, E. C., 1926 The temperature-effective period—the key to eye-facet number in Drosophila. J. Exp. Zool. **46**: 317–332.

1931 Temperature and gene expression in Drosophila. J. Exp. Zool. **59**: 1–28.

DUBININ, N. P., 1929 Allelomorphentreppen bei *Drosophila melanogaster*. Biol. Zbl. **49**: 328–339.

GOLDSCHMIDT, R., 1931 Die entwicklungsphysiologische Erklärung des Falls der sogennanten Treppenallelomorphe des Gens scute von Drosophila. Biol. Zbl. **55**: 535–554.

1938 Physiological Genetics. ix+375 pp. McGraw-Hill Book Co. New York and London.

HARNLY, M. H., 1936 The temperature-effective periods and the growth curves for length and area of the vestigial wing in *Drosophila melanogaster*. Genetics **21**: 84–103.

HARNLY, M. H., and HARNLY, MARIE L., 1936 The effects of the gene on growth and differentiation as shown by the temperature responses of pennant and its heterozygote in *D. melanogaster*. J. Exp. Zool. **74**: 41–59.

IVES, P. T., 1935 The temperature-effective period of the scute-1 phenotype. Proc. Nat. Acad. Sci. **21**: 646–650.

LI, J. C., and TSUI, Y. L., 1936 The development of vestigial wings under high temperature in *Drosophila malanogaster*. Genetics **21**: 248–263.

LUCE, W. M., 1931 The temperature-effective period in infra-Bar and its heterozygotes. J. Exp. Zool. **59**: 467–498.

MARGOLIS, O. S., 1935 Studies on the Bar series of Drosophila. I. The effect of the gene vestigial on facet number in Bar. Genetics **20**: 156–171.

1935 II. The temperature-effective period for facet determination in Bar and Bar-vestigial. Genetics **20**: 207–222.

PAYNE, F., 1920 Selection for high and low bristle number in the mutant strain, "reduced." Genetics **5**: 501–542.

PLUNKETT, C. R., 1926 Interaction of genetic and environmental factors in development. J. Exp. Zool. **46**: 181–244.

1932 Temperature as a tool in phenogenetics: methods and results. (Abstract). Proc. Sixth Int. Cong. Genetics **2**: 158–160.

POWSNER, L., 1935 The effects of temperature on the duration of the developmental stages of *D. melanogaster*. Physiol. Zool. **8**: 474–520.

RIEDEL, HILDA, 1934 Der Einfluss der Entwicklungstemperatur auf Flügel-und Tibialänge von *Drosophila melanogaster* (wild, vestigial und die reziproken Kreuzungen). Arch. f. Entw-Mech. **132**: 463–503.

ROBERTSON, C. W., 1936 The metamorphosis of *Drosophila melanogaster* including an accurately timed account of the principal morphological changes. J. Morph. **59**: 351–399.

STANLEY, W. F., 1935 The effect of temperature upon wing size in Drosophila. J. Exp. Zool. **69**: 459–495.

STURTEVANT, A. H., and SCHULTZ, J., 1931 The inadequacy of the sub-gene hypothesis of the nature of the scute allelomorphs of Drosophila. Proc. Nat. Acad. Sci. **17**: 265–270.

A QUANTITATIVE STUDY OF GENIC EFFECTS ON GUINEA-PIG COAT COLORS[1]

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INTRODUCTION

THE problem of the manner in which genes produce their effects may be favorably approached in a study of the coat color of mammals, as there is reason to believe that the chain between gene and phenotype is here relatively short. The persistence of color in mutant spots and in transplantation experiments indicates that the pigment is determined locally, and the processes concerned continue throughout life, as hair is constantly being shed and replaced. The guinea-pig is particularly good material for work on pigmentation because of the studies of WRIGHT (1916, 1917, 1925, 1937), who described the effects of its color factors in different combinations and attempted to determine from those the simplest possible scheme for the interactions of the gene products involved. This provides a background for further work, an hypothesis which may be tested.

The main color factors of the guinea-pig fall into three groups. The first group has to do with the restriction of coloration to a piebald pattern on a white ground, and is represented by the pair *S*, *s*, and minor modifiers. The second group has to do with the primary qualitative differentiation of pigment, that between yellow and darker colors. It is represented by *E* (black, sepia, brown, etc.), e^p (tortoise), *e* (red, yellow, cream, etc.), and by *A* (agouti), a^r (dark agouti), *a* (solid black, etc.). The third group of factors varies the intensity of color in the sepia or yellow series, or both, but not their distribution. This group includes the albino series (*C*, c^k, c^d, c^r, c^a), affecting the intensity of both yellows and sepias; *P*, *p* and *B*, *b*, affecting only the sepia series; and *F*, *f* affecting yellows in all combinations but sepias only in combination with *p p*. This paper deals mainly with the third group of factors and somewhat with the qualitative differences between the pigments produced by *E* and *e*.

The colored materials of the hair are melanins, proteins of unknown composition, probably derived from tyrosin, dioxyphenylalanin, or allied

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substances. They are found widely distributed in both vertebrates and invertebrates, although it is highly probable that all melanins are not identical. They are characterized by extreme inactivity and generally by dark color. A description of them and their properties may be found in FÜRTH (1924) and SAMUELY and STRAUSS (1922). The exact manner of formation of these substances is not known, but since the discovery of tyrosinase by BERTRAND (1896), it has been generally accepted that a substrate, or chromogen, and an oxidative enzyme are involved. BLOCH (1917) found that the enzyme in human pigment-forming cells reacts to dioxyphenylalanin (dopa) and not to tyrosine, and called it dopaoxidase. The results of W. L. RUSSELL (1939) indicate that dopaoxidase is effective in guinea-pig pigmentation also. The studies of RAPER (1928, 1932) have done much to further our knowledge of the possible stages in the reaction leading from tyrosine to melanin. Although knowledge of the concentration of any component of the pigment reaction would be of value in a quantitative study of gene action, one obviously essential step is a determination of the actual quantities of end-product present in various genotypes.

GENERAL METHODS

Quantitative measurements of the melanins in hair are made difficult by their inactivity and the great similarity of their reactions to those of the hair protein, keratin. The possible mechanisms for separating these two components of mouse hair are discussed by EINSELE (1937). He concludes that acid hydrolysis is most suitable, as both keratin and melanin are alkali soluble, but although keratin may be hydrolyzed by sufficiently long treatment with concentrated acid, the melanin is unaffected by this treatment. In view of the distinction made by GORTNER (1912) between acid-soluble and acid-insoluble melanins, it should be noted that EINSELE's statement concerning the insolubility of mouse melanin depends on the fact that the color obtained in the acid hydrolysate is always pale yellow, showing no correlation with the amount of melanin in the hair treated. From this he concludes that no melanin has been dissolved in the acid. By this same reasoning the same conclusion holds for guinea-pig melanin. The acid hydrolysate here is always straw-colored, and no differences in color are found corresponding to the intensity or type of color in the hair, even in the extreme cases, black, red and white. This method may be used for isolating melanin for quantitative comparison of various types on the assumption that if there is any effect of the acid not detected by the color of the hydrolysate, it is approximately the same for all types. EINSELE's method of acid hydrolysis was adopted for the isolation of guinea-pig melanin. However, the lightness and flocculence of the dilute yellows has made their separation from an acid hydrolysate so difficult that results

for these types by this method are not sufficiently accurate, and it has seemed advisable to measure the concentration in yellows by some other method.

As reported by DURHAM (1904), the yellow pigment in guinea-pig hair is much more soluble in alkali than is the sepia. I found the yellow pigment completely and rapidly soluble in cold dilute alkali, while the sepia dissolves very slowly and never becomes completely dissolved by this treatment. This high solubility of yellows in alkali has made possible colorimetric comparisons of the color obtained from the various yellow genotypes, providing the desired substitute for the acid hydrolysis of these types.

EINSELE followed his isolation of melanin by a determination of the percent of the total weight which it formed in each type. He obtained consistent results when the percent weight was two or over, but not below this figure. Unfortunately, the weights of melanin are less in the guinea-pig than in the mouse, and in the pale sepias (*E p p*) and yellows (*e e*), the amount seems to be too low for accurate determinations. A more accurate method useful for these types has been developed in this study. Instead of weighing the isolated melanin, the amount of $KMnO_4$ necessary to oxidize it has been determined. Permanganate oxidation of melanin was first used by BACH (1908) to measure quantitatively the product obtained by the action of tyrosinase on tyrosine, and was considered by him an accurate indicator.

Thus it has been possible to find three methods for measuring melanin concentration in the hair, although none is suitable for all types:

1. The colorimetric comparison of alkaline solutions of the yellow series.
2. The percent weight of melanin in *P*-sepias as determined by the valuable technique of EINSELE (1937).
3. The permanganate oxidation values of the entire sepia series and the higher members of the yellow series as determined for pigments isolated by the EINSELE technique.

Certain procedures are common to all the methods used in this study, and they may be mentioned here. In all cases, hair was collected from the middle part of the back of two to three week old animals. This hair was removed down to an approximately equal length of base by hair clippers or scissors. Variations in total length of hair combined with variations in depth of color along the hair are an undoubted but unavoidable source of error in these results. The age of two to three weeks seemed advisable as guinea-pigs younger than this did not provide enough hair and the coat color of older animals has been subjected longer to environmental influences. In all cases, two samples from the same animal were used in order to detect gross errors in technique. Unless otherwise stated, no data were

accepted if one of the samples was lost or gave a difference between the two samples for one animal exceeding 25 percent, a very rare occurrence. In all cases, results are classified in two ways, according to genotype and according to a grade of color assigned to each animal. The genotypes were determined by DR. WRIGHT, using as criteria the genotype of the parents, the animal's own color, and, in certain cases, breeding tests. The animals used came from an extensive series of experiments conducted by DR. WRIGHT for the purpose of producing animals of as great a variety of known genotypes with respect to color as possible. The grades were assigned by DR. WRIGHT within one or two days after birth by comparison with a standard set of hair samples, arranged in steps of just perceptible increase in depth of color (WRIGHT 1927). There are thirteen steps in the yellow series, twenty-one in the sepias. The colors change somewhat with age, but there is no appreciable change between birth and three weeks.

COLORIMETRIC METHOD FOR YELLOWS

As stated above, the yellow pigment of guinea-pig hair is soluble in dilute alkali. A measure of the relative amount of pigment in two different genotypes may be obtained by extracting the pigment from equal weights of hair of each type in equal amounts of alkali under identical conditions. The intensity of color in the filtered solutions may then be compared by matching the two solutions in a colorimeter. The color seen in the colorimeter depends upon the intensity of the color in the solution, and upon the height of the column of liquid through which the light passes. In matching a standard color the height of the column of liquid will be shorter for a dark shade than for a pale one. According to Beer's law, the product of height of liquid 1 times intensity of color 1 is equal to the product height 2 times intensity 2 and the ratio of intensities of two samples is, therefore:

$$\frac{\text{intensity 1}}{\text{intensity 2}} = \frac{\text{height 2}}{\text{height 1}}.$$

In my experiments I compared the intensity of color from each hair sample with that of a standard sample from $e\ e\ c^d\ c^d$, yellow of grade seven. It was necessary from time to time to replace the standard because of loss of small amounts in transferring to the colorimeter. When this was necessary, a group of samples from $e\ e\ c^d\ c^d$ hairs were made up, and that which agreed most closely with the old standard was taken for the new one. This method of selecting standards might lead, if the originals faded before they were matched, to a gradual reduction in the intensity of the standard. The possibility of a serious shift was avoided by the use of one genotype only, $e\ e\ c^d\ c^d$, those selected always being of grade 7. Six standards were

used in all, the largest number of ratios being determined with the first and fourth in the series. Table 1 shows the ratios obtained with these two standards, classified by grade.

TABLE 1

WRIGHT'S GRADE	STANDARD 1		STANDARD 4		DIFFERENCE 4−1
	MEAN	NO.	MEAN	NO.	
0	.108	2	.107	2	−.001
1			.130	1	
2	.238	6	.299	1	+.061
3	.281	8			
4	.503	8	.634	2	+.137
5	.578	8	.688	4	+.110
6	.714	3	.871	3	+.157
7	.956	16	1.029	9	+.073
8	1.130	1			
9			2.308	1	
10	3.010	8	2.940	6	−.070
11	2.834	4	3.259	5	+.425

Among the eight comparisons which can be made, the ratio is higher in six and lower in two, the average ratio of fourth to first being 1.12. This difference is probably significant (probability .04 by STUDENT'S method), but it is not certain that it indicates a change in the standard as different animals were used in the two series. In any case, it is not serious (less than one-third of a grade), the average increment from one grade to the next being about 38 percent.

TABLE 2

Colorimeter comparison with standard of yellow solutions obtained with different concentrations of alkali.

ANIMAL	CONCENTRATION OF ALKALI (NORMALITY)								
	.25	.5	1.5	2.13	2.75	3.38	4.00	5.25	6.50
1	.895	1.027	.886	.994	.809	.943	.707	1.076	.863
2	.729	.763	.738	.618	.703	.747	.535	.692	.607
3	.808	.885	.950	.888	.875	.978	.925	.938	.905
4	.816	.820	1.158	.910	.831	.780	.865	.902	.840
5	.933	.846	.871		.988	.857	.733	1.008	.813
6	.803	.855	.942	.877	.810	1.013	.918	.857	.847
Mean	.831	.866	.924	(.857)	.836	.886	.781	.912	.813

The effects of various factors on the alkaline solutions were determined. The effect of concentration of alkali was tested, using concentrations from .25/N–6.5/N NaOH. Nine equal samples were taken from each of six ani-

mals and dissolved in equal volumes of NaOH of varying concentration. As can be seen from table 2, no significant changes with concentration appear when these are compared with the standard.

The effect of long boiling was also tested and found to be negligible, providing the original volume was restored before each ratio of boiled to standard was determined. Two 100 cc colored solutions were boiled 3.5 hours with color determinations every ten minutes. The results are given in table 3.

TABLE 3

Effect of long boiling on alkaline solutions of yellow pigment.

MINUTES BOILED	COLORIMETER COMPARISON WITH STANDARD		MEAN
	SOLUTION 1	SOLUTION 2	
10	1.005	.984	.995
20	1.073	1.042	1.058
30	.933	1.003	.968
40	.863	1.060	.961
50	.792	1.040	.916
60	.883	1.045	.964
70	.874	1.073	.974
80	.951	1.109	1.030
90	.869	1.065	.967
100	.999	1.073	1.036
110	.878	1.155	1.017
120	1.007	1.073	1.040
130	.923	1.289	1.106
140	1.071	1.284	1.178
150	.850	1.195	1.023
160	1.082	1.107	1.095
170	1.084	1.185	1.121
180	.924	1.185	1.055
190	.891	1.087	.989
200	.949	1.074	1.012
210	.975	1.165	1.070

The effect of electric light on these alkaline pigment solutions was tested by placing six samples of a homogeneous solution 12 inches from a 60 watt-110 volt lamp for periods varying from 12 to 96 hours and comparing these with a control sample kept in darkness. The ratios are given in table 4.

Similar results were obtained with two other samples at six to 48 hours. As the hair (after collection) and the solutions are kept in darkness except during tests, it is probable that no appreciable fading occurred.

The final method adopted was to wash the hair to be tested in cold ether to remove at least the major part of the fat which might alter the color. From this hair, after drying, two 100 mg samples (to the nearest milligram)

TABLE 4

Effect of light on alkaline solutions of yellow pigment.

HOURS IN LIGHT	COMPARISON TO CONTROL
0	1.010
12	1.005
24	1.000
36	1.005
48	1.013
72	.990
96	1.000

were removed. Each was placed in 10 cc of .5 or .25/N NaOH for five days. The hair was then filtered off and the resulting colored solution compared in a colorimeter (Klett Biometer) with the standard, and the ratio of intensity of tested sample to that of the standard recorded. The two samples from one animal were also checked against each other. Before any comparisons of intensities with different genotypes were made, the ratios were corrected by subtracting the slight amount of color (.120) obtained from white (presumably fat and hydrolyzed keratin). The accuracy of this

TABLE 5

Mean colorimetric values of the yellow genotypes.

GENOTYPE	MEAN WRIGHT'S GRADE	MEAN COLORIMETRIC VALUE	S.E.	NUMBER
e e C- F-	10.33	2.866	± .114	27
e e c^k c^k F-	6.92	.949	± .055	10
e e c^k c^d F-	7.00	1.031	± .150	6
e e c^k c^r F-	4.89	.616	± .048	13
e e c^k c^a F-	5.00	.489	± .025	4
e e c^d c^d F-	6.93	1.150	± .082	23
e e c^d c^r F-	4.16	.593	± .046	19
e e c^d c^a F-	4.20	.484	± .036	18
e e c^r c^r F-	0.00	.133	± .017	2
e e C- f f	7.00	.888	± .053	7
e e c^d c^d f f	2.46	.280	± .031	10
E- C- f f p p	3.00	.469	± .037	2

method may be indicated by the variability of the ratios between two samples from the same animal. The ratio of second to first in 152 animals was 1.003 with a standard deviation of ±.126. About one-twentieth of this variability was due to errors in colorimetry. In the determinations of each ratio, three independent readings were made with the colorimeter. The mean ratio of the second of these to the first is .992 with a standard deviation of ±.072. It is necessary to multiply this standard deviation by

$\sqrt{1/6}$ in order to make it comparable to the standard deviation of the ratio of two samples from the same animal (each the average of three colorimetric comparisons), giving .029 or 23.3 percent of .126, making the variance 5.5 percent that of the former ratio. The remaining error in the ratio of two samples from the same animal must be due to variations in the pigment in the hair or in the preparation of the material.

TABLE 6

Mean colorimetric values of Wright's color grades for yellows.

WRIGHT'S GRADE	MEAN COLORIMETRIC VALUE	OBSERVED STANDARD ERROR	STANDARD ERROR CALCULATED FROM COEFF. VARIABILITY	NUMBER
0	0.120	±.016	±.017	3
1	0.122	—	±.031	1
2	0.239	±.026	±.030	4
3	0.357	±.030	±.026	12
4	0.552	±.027	±.030	22
5	0.627	±.046	±.038	17
6	0.778	±.049	±.056	12
7	1.039	±.047	±.045	34
8	1.403	±.197	±.144	6
9	2.237	±.117	±.397	2
10	2.872	±.156	±.186	15
11	3.020	±.199	±.253	9
12	2.654	—	±.667	1

The results obtained with this method are given in table 5 and figure 1 according to genotype and according to WRIGHT's grades in table 6 and figure 2.

It was found that WRIGHT's grades for the *C* series, as given in this 1927 paper, could best be compared with the colorimetric values by regarding the former as geometric rather than as arithmetic steps. The transformation was accomplished by plotting the logs of the colorimetric values (CV), corrected for the color from white hair, against the grades (G) and finding the best straight line through the points, weighting the means by their generalized standard errors determined from the weighted mean coefficient of variability. This arrangement of the data is shown in figure 2. The formula for the line, as determined by the weighted least square method, is:

$$\text{Log (CV- .120)} = \bar{9}.032 + .135\ \text{G}.$$

The standard error of the slope of this line is ±.005. The results with this transformation of the 1927 grades are given in table 7 and figure 3. This transformation seems justifiable, as it seems probable by the Weber-Fechner law that steps equally spaced to the eye show equal percentage increments rather than absolute increments.

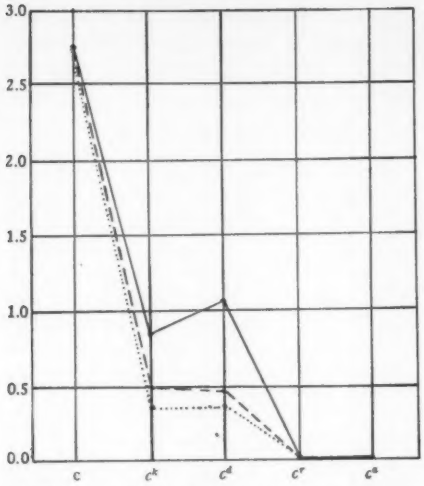

FIGURE 1.—The colorimetric values (ordinate) of various yellow genotypes (abscissa). The solid line shows the values obtained with the homozygote of the allele indicated below the graph; the dash line represents those obtained with its heterozygote with c^r; and the dotted line those with its heterozygote with c^a.

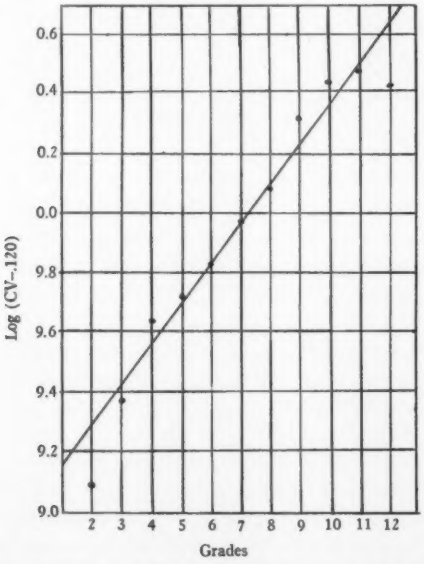

FIGURE 2.—Logarithm of (CV- .120) (ordinate) for each of WRIGHT'S grades for yellow (abscissa).

TABLE 7

Comparison of Wright's grades for yellow genotypes (transformed to a geometric scale) with the mean colorimetric values for the same genotypes.

GENOTYPE	WRIGHT'S GRADE (1927)	TRANSFORMED WRIGHT'S GRADE	CORRECTED COLORIMETRIC MEAN
C-	10.6	2.78	2.75
$c^k c^k$	7.1	.92	.83
$c^k c^d$	7.2	.94	.91
$c^k c^r$	4.6	.42	.50
$c^k c^a$	4.6	.42	.37
$c^d c^d$	7.0	.89	1.03
$c^d c^r$	4.1	.36	.47
$c^d c^a$	4.2	.37	.36

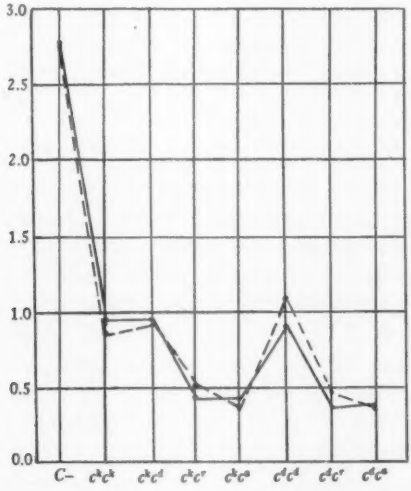

FIGURE 3.—Comparison of the colorimetric values with WRIGHT'S grades transformed to a log scale. The solid line connects the transformed WRIGHT'S grades, the dash line the corrected colorimetric values.

PERCENT WEIGHT OF MELANIN IN SEPIAS

The method used for this study was the extremely valuable EINSELE technique (1937). This consists of separating the acid-insoluble melanin from the keratin by acid hydrolysis which breaks up the keratin and appears to leave the melanin unmodified. To prepare the hair for this treatment it is necessary to wash it with warm ether for a number of days to remove the fat particles which, upon acid hydrolysis, would form a ring around the sides of the condenser and adsorb a significant part of the

melanin granules. In place of the Jena glass and block-tin condenser used by EINSELE, an all metal apparatus was used in these experiments. This consisted of a rectangular galvanized-tin jar, coated with block-tin to prevent the entrance of impurities into the ether. Inside this cylinder the hair rested in nichrome baskets on a set of ten nichrome (ether resistant) screen trays, supported on a block-tin coated galvanized-tin rack. A five centimeter layer of ether was maintained in the bottom of this jar. The top of the cylinder was an inverted truncated pyramidal chamber, through which cold water ran continuously, with a drip plate on its lower surface the same size as the nichrome trays and directly above them. The ether vapor recondensed on this plate, and thus fell directly onto the hair. Ether leakage was prevented by a Bruhl wax seal and by equalization of the pressure inside the ether bath with that of the atmosphere by inserting an ordinary open condenser into the top of the bath, allowing for escape of air but preventing escape of ether. Heat was applied to the bath by two 100 watt electric bulbs enclosed in a separate chamber fitting beneath the main jar.

The procedure followed was to wash the hair (about .750–1.000 grams from each animal) in 0.1 percent sodium lauryl sulphate for one hour. This high molal salt of a fatty acid is sold commercially as "Dreft," and has the saponifying qualities of soap, but is neutral in reaction (a feature necessary for washing the alkali-soluble yellows), and can be used cold, which is further guarantee of retention of the yellow pigment. (I am grateful to DR. D. G. STEELE for this suggestion concerning technique.) The Dreft was decanted and followed by four washes with hot distilled water. Each sample was transferred to a nichrome basket and dried in an oven at 90–100°C. The dried cooled hair was placed in the ether bath, two samples to a tray, and washed with ether. By daily weighing tests, it was found that all of the fat was removed in four days, so five days was accepted as a safe standard time for washing with ether. On removal from the bath, each sample was divided into two samples and dried. The weight of the dry hair was determined (mean weight .377 grams) and the hair was transferred to an all-glass reflux condenser and boiled for one hour with 50 cc 6/N HCl. At the end of this hydrolysis, the hair was completely broken up and the melanin found as free granules. Addition of 25 cc of 95 percent alcohol to the hydrolysate was found to facilitate separation of the melanin granules from the liquid. (I am grateful to DR. STEELE for this suggestion also.) This separation was accomplished by centrifuging, ten minutes at 3600 rpm. being sufficient for practically all types. The liquid was decanted, replaced by distilled H_2O, and the melanin again centrifuged out. This was done four times, and tests of the fourth wash were always negative for chloride, indicating that all of the acid, and consequently all of the dis-

solved keratin, had been removed. The precipitate was then transferred to weighing bottles, dried, and weighed. The final data recorded were the mean percentage weights of melanin in the two parts of each hair sample. The two samples from each animal serve as a check on gross errors. This method was found to be satisfactory only for *P*-sepias, as *p*-sepias and yellows have smaller weights of pigment, and the error is so great as to make the results of little value.

This determination was used for two *P*-sepia genotypes, in order that the results might be compared with, and act as a check upon, the numbers obtained for these genotypes by the permanganate method. The results are given in table 8. The percent of acid-removable substances in white was also determined by boiling white hair samples with black of known melanin content and determining the increment due to substances adsorbed from the whites. This value, the percent weight of melanoidins and other impurities resulting from acid hydrolysis of hair, was subtracted from each mean to give the true percent content of melanin. By this method c^d c^a *E F P* has 47.9 percent as much pigment as *C-E F P*.

TABLE 8

Percent weight of melanin in two sepia genotypes.

GENOTYPE	MEAN	S.E.	S.D.	NUMBER	TRUE MEAN
C-E-F-P-	3.34	±.21	±.67	10	2.86
c^d c^a *E-F-P-*	1.85	±.13	±.42	10	1.37
White	0.48	±.09	±.27	9	0.00

THE PERMANGANATE METHOD

A smaller difference between the two samples from one animal and among all the animals of the same genotype was found with a modification of the method given in the foregoing section. In this method the isolated melanin, and the small amount of distilled water left after the fourth decantation after washing, were transferred to a 150 cc beaker, acidified with 2 cc concentrated H_2SO_4, and heated to the boiling point. The hot mixture was titrated against .005–.01/N $KMnO_4$ until the addition of .5 cc left a pink color after one minute of stirring. With samples of high melanin content the amount added was increased to 1 cc to compensate for volume differences. BACH (1908) states that it is sufficient that the black melanin mixture be titrated with permanganate just to decolorization in order to obtain consistent results. I attempted to use this method, but could not decide exactly when the melanin reached "decolorization." It continued to reduce potassium permanganate rapidly for a time after losing all color, and samples left for several hours always lost their pink color no matter how great an excess of permanganate had been added. The explanation

of this phenomenon is not known. Since BACH apparently titrated his samples only until they were decolorized, he did not encounter this difficulty. The decolorization point may have been easier to determine in his material, as the melanin was undoubtedly purer and in finer particles, being made artificially by the action of tyrosinase on tyrosine in solution. As my material was oxidized more, rather than less, than BACH'S, it seems probable that the entire amount of pigment is measured by the permanganate oxidation at all points in the scale of intensity of color.

The timed end-point makes this method liable to considerable subjective error, but every attempt has been made to keep this at a minimum. The policy followed was to keep the operator ignorant of the genotype being tested and the result obtained for a second sample from the same animal until titration was completed, to keep lighting and temperature as constant as possible, and to seek corroboration of the end-point whenever feasible. As considerable calculation is necessary before the final value is obtained, there is little danger of trying to match samples.

Potassium permanganate can not be made up to a desired normality, but its normality can be accurately determined. The policy was, therefore, to make up $KMnO_4$ somewhere near .005–.01/N, and determine the normality with $Na_2C_2O_4$. Comparable figures for different $KMnO_4$ solutions were obtained by expressing the final results in a form called for convenience the permanganate number:

$$(PN) = \frac{\text{cc } KMnO_4 \times N\ KMnO_4 \times 100}{\text{grams hair}}.$$

Tests were made with .016/N and .038/N $KMnO_4$ and the results obtained this way were much more different from those obtained within the limits .005–.01/N $KMnO_4$ than any of those were from each other. This seems to indicate an effect of concentration of permanganate, which should be further investigated. The permanganate numbers with the higher normality permanganate were too high in all genotypes tested. Analyses of the data show no significant effect of this factor in the range from .005–.01/N, and the data obtained with higher concentrations were discarded.

A second difference from the method described earlier was the addition of 100 mg of black of known permanganate value to each sample before boiling. The addition of black was found by EINSELE to be helpful in dealing with light pigments which become adsorbed on the melanin granules of the black and thus centrifuge out of the solution easily. The samples added were obtained by combining all of the hair from the backs of a number of black guinea-pigs, and working them into a homogeneous mixture. After washing in the ether bath, this hair was divided into 100 mg samples (about 200 samples from one batch of hair) and determinations made of

TABLE 9

Coefficients of variability obtained with the permanganate and percent weight of melanin methods.

GENOTYPE	COEFF. VAR. (PERMANGANATE NUMBER)	COEFF. VAR. (% WEIGHT MELANIN)
C-E-F-P-	10.5	19.2
c^a c^a *E-F-P-*	18.5	21.6
White	16.8	52.1

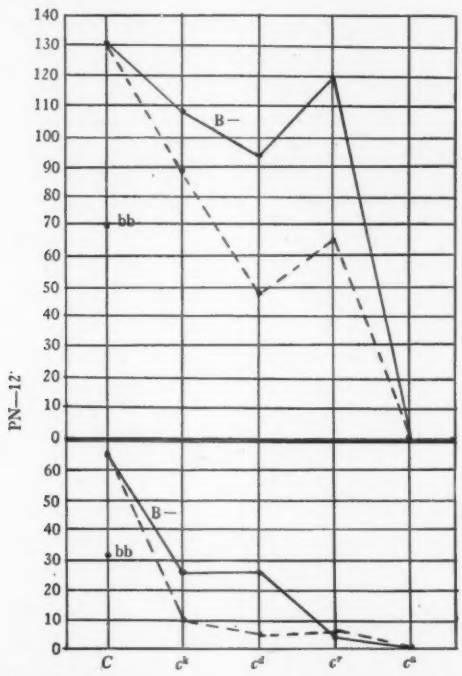

FIGURE 4.—The corrected mean permanganate number for various sepia and brown genotypes. The upper figure shows the darker sepias and browns, the lower the pale sepias and browns. The solid line shows the values obtained with the homozygote of the allele indicated below the graph, while the dashed line indicates those obtained with its heterozygote with c^a.

the permanganate numbers of ten of these, chosen at random. The mean amount of $KMnO_4$, in terms of mols, used up by these samples was then subtracted from the total permanganate used by each sample. The amount which this addition of black hair adds to the variability of the final permanganate values may be estimated from the standard deviations of the mean permanganate numbers of the blacks for combination. Two series

TABLE 10

Permanganate numbers observed for each of the genotypes tested.

C SERIES	PERMANGANATE NUMBERS 0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–99	100–109	110–119	120–129	130–139	140–149	150–159	160–169	NO.	MEAN	S.E *
E- F- P- B-, darker sepias																				
C-											1				3	2	1	7	143	± 9
$c^k c^k$										1			1		1			3	120	± 12
$c^k c^r$																	1	1	166	± 26
$c^k c^a$											1							1	100	± 17
$c^d c^d$											2							2	106	± 13
$c^d c^r$									1	1		2	2		1		1	8	121	± 7
$c^d c^a$					1	3	3			1								8	60	± 4
$c^r c^r$												1	1			1		3	131	± 12
$c^r c^a$						1	1	1	2	1								6	77	± 6
E- F- $p^p p^p$ *B-*, pale sepias																				
C-								1										1	76	± 14
$c^k c^k$			3	3	1		1											8	37	± 3
$c^k c^d$			1															1	23	± 7
$c^k c^r$			1															1	29	± 7
$c^k c^a$		3	3															6	21	± 3
$c^d c^d$				2														2	37	± 6
$c^d c^r$		2	4	1														7	21	± 2
$c^d c^a$		3	1															4	17	± 3
$c^r c^r$		4	1															5	16	± 3
$c^r c^a$	1		2															3	18	± 3
E- F- P- b b, darker browns																				
C-								2	2	1								5	81	± 7
$c^k c^a$									1									1	83	± 15
E- F- $p^p p^p$ *b b*, pale browns																				
C-				1	1													2	43	± 7
e e F-, yellows																				
C-			1	4	2													7	38	± 3
$c^k c^k$		1																1	18	± 5
$c^k c^r$		2																2	17	± 3
$c^k c^a$		1																1	18	± 5
$c^d c^d$		2	3															5	21	± 2
$c^d c^r$	1	3	3															7	17	± 2
$c^d c^a$		1	2															3	21	± 3
e e f f, pale yellows																				
C-		3	1															4	18	± 3
$c^d c^d$	1	3																4	12	± 2

* (The standard errors were determined on the basis of a constant coefficient of variability, the weighted mean coefficient of variability of all types involved. In taking all of the coefficients of variability, allowance was made for the variability of the 100 mg of black hair added to each sample, by adding to the mean permanganate number for each type an amount equal to the product of the mean fraction of the total weight of the sample constituted by this black, and the weighted mean permanganate number of this black. The actual amount added was 26.)

were used, the first having a mean value of 108 with a standard deviation of 8, the second a mean value of 146 with a standard deviation of 4. Allowance for this factor was made in the determination of the standard errors

TABLE 11

Permanganate numbers observed for each of Wright's grades.

WRIGHT'S GRADES	0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–99	100–109	110–119	120–129	130–139	140–149	150–159	160–169	NO.	MEAN	S.E.*
Sepias																				
0–1	7	5	3															15	12	± 1
2		8	1															9	15	± 2
3		2	4															6	19	± 2
4			2	1														3	27	± 4
5		2	3															5	21	± 3
6			2	1														3	32	± 4
7				1	1													2	30	± 5
8			2	1														3	32	± 4
9				1			1											2	50	± 6
10																				
11																				
12					1			1										2	62	± 7
13						2	1											3	56	± 6
14							3											2	64	± 6
15						1	1	1	2	1								6	75	± 5
16										1								1	99	±15
17									1		1							2	96	±10
18											1							1	103	±15
19										1	1	2	1				1	6	120	± 7
20										1	1	1	1		1			5	115	± 7
21													2		4	2	1	9	144	± 7
Yellows																				
0–1	7	5	3															15	12	± 1
2	1	2																3	11	± 3
3	1	1	1															3	14	± 3
4		3	2															5	19	± 3
5		1	1															2	20	± 4
6		2																2	17	± 5
7		6	5															11	20	± 2
8				1														1	30	± 9
9			1	1	1													3	37	± 5
10				2														2	36	+ 6
11																				
12					1													1	42	± 9

* (Standard errors were determined as in Table 10.)

of all types tested. This method not only made possible tests for pale sepias and yellows which otherwise would not have precipitated well, but also gave accurate tests of white which indicate the part of each permanganate titration due to melanoidins (darkish materials of unknown composition which appear in small concentrations with acid hydrolysis of keratin) and other impurities. This method was more accurate than the weighing method for the lower concentrations, as can be seen from the coefficients of variability of the means for the genotypes tested by both methods, as given in table 9.

Even with this method only the higher genotypes of the pale sepia and yellow series can be taken seriously, but they at least give the approximate location of these types on the total scale. The results obtained with this method are given in table 10 and figure 4 according to genotype, and according to WRIGHT'S grades in table 11 and figure 5. In comparing the

permanganate numbers to WRIGHT's grades the same procedure has been followed as with the colorimetric data. That is, WRIGHT's grades are considered as geometric increments and the permanganate numbers as arithmetic. The exact relationship between the two scales was found by plotting the log of the mean permanganate number for each of WRIGHT's grades against the grades (see figure 5). The permanganate numbers were corrected for adsorbed impurities by subtracting 12 (the mean permanganate number of all samples of grades 0 to 1 in either series, these having only

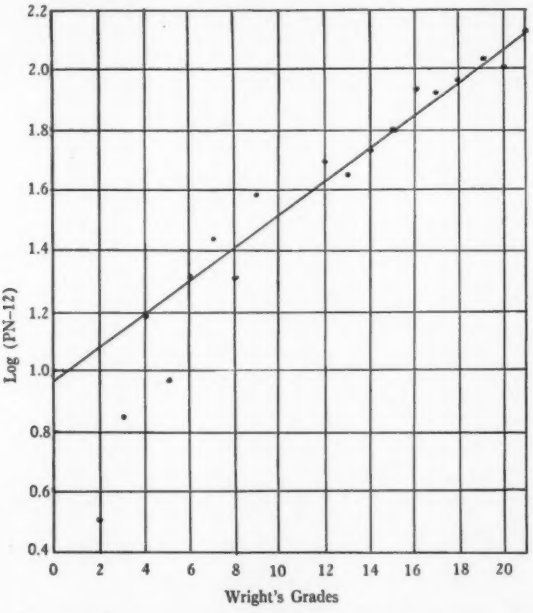

FIGURE 5.—The log of the mean permanganate number for each of WRIGHT's color grades for sepias, plotted against the grades, with the calculated best straight line through all grades from 2–21.

the faintest tinge of color) from all the means. The formula for the best straight line through all grades from 2–21, as determined by the least squares method, with values weighted by their standard errors, is:

$$\text{Log (PN} - 12) = .953 + .056\ \text{G.}$$

The standard error of the slope of this line is $\pm .0025$. A comparison of WRIGHT's grades for sepia genotypes, transformed according to this formula, with the mean permanganate numbers observed for each genotype is given in table 12 and figure 6. Good agreement is found in all cases ex-

TABLE 12

Comparison of Wright's grades for sepia genotypes (transformed to a geometric scale) with the mean permanganate numbers for the same genotypes.

C SERIES	WRIGHT'S GRADE (1927)	TRANSFORMED WRIGHT'S GRADE	CORRECTED MEAN PERM. NUMBER
	Sepias		
C-	21.00	135	131
$c^k c^k$	20.10	120	108
$c^k c^r$	20.46	126	154
$c^k c^a$	18.53	98	88
$c^d c^d$	16.88	79	94
$c^d c^r$	19.09	105	109
$c^d c^a$	14.02	55	48
$c^r c^r$	20.09	120	119
$c^r c^a$	15.49	66	65
$c^a c^a$	0.00	0	0
	Browns		
C-	15.63	67	69
$c^k c^a$	13.60	52	71

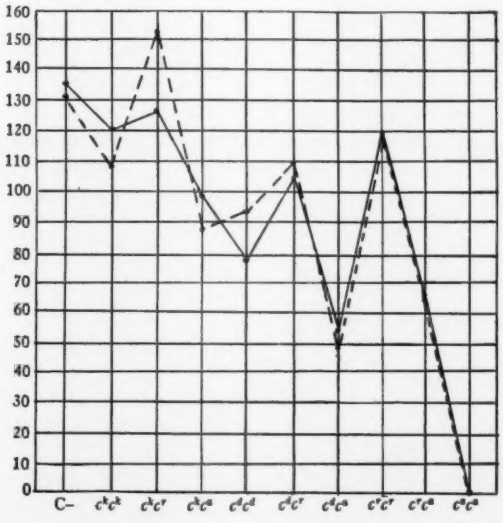

FIGURE 6.—A comparison of WRIGHT'S grades, transformed to a log scale, for each darker sepia genotype (solid line) with the mean corrected permanganate number (dash line).

cept $c^k\ c^r$, where the observed permanganate number, based on a single animal, has a standard error of ± 26, sufficient to account for the aberration.

Comparisons between the two samples from the same animal gave a mean difference of 8 ± 1 (11 in darker sepias, 9 in browns, 5 in pale sepias, and 6 in yellows), which gives some idea of the experimental error. The relative undependability of the lower grades of pale sepias is brought out by these figures and also shows up clearly in table 10.

A further experiment with this method was the comparison of sepia litter mates of genotypes $c^d\ c^a$ and $c^r\ c^a$, in an attempt to determine the reality of the darker color in $c^r\ c^a$, which appears in WRIGHT's grades and in my data, by eliminating as far as possible strain differences which might produce this result. These results are given in table 13. They include data

TABLE 13

Litter mate comparisons of permanganate number of $c^d\ c^a$ and $c^r\ c^a$ sepias.

LITTER	AGE	MEAN P.N. $c^d\ c^a$	MEAN P.N. $c^r\ c^a$	$c^r\ c^a - c^d\ c^a$
1	2–3 weeks	56	83	+27
2	2–3 weeks	62	79	+17
3	2–3 weeks	47	82	+35
4	11 months	79, 71 } 75	86	+11
5	2 weeks	99	92, 83 } 88	−11
			Mean Difference	+16 ±8

on older animals as well as the two–three weeks old guinea-pigs used in the regular experiments. The significance of the difference between the two genotypes can be tested by STUDENT's method, using averages where there is more than one of a genotype in a litter. The result ($t = 2.0$, $n = 4$) yields a probability of .12 of a greater difference by accidents of sampling. The difference can thus be considered merely suggestive of a greater amount of pigment in $c^r\ c^a$ than in $c^d\ c^a$.

It is important to test the agreement between the permanganate method and the percent weight of melanin method. There is 36.6 percent as much melanin in $c^d\ c^a\ E\ F\ P$ as in $C\ E\ F\ P$ by the permanganate method, 47.9 percent by the percent weight method. The difference between these ratios is 1.6 times its standard error, and is thus not significant.

$$\left(E \text{ ratio} = E_{x/y} - \frac{\overline{X}}{\overline{Y}}\sqrt{\left(\frac{E_x}{X}\right)^2 + \left(\frac{E_y}{Y}\right)^2}, \quad E_{r_1-r_2} = \sqrt{(E_{r_1})^2 + (E_{r_2})^2}\right)$$

$$r_{r_1} - r_2 = .113, \quad E_{r_1} = .040, \quad E_{r_2} = .057, \quad E_{r_1-r_2} = .070.$$

INTERPRETATION

C series

As may be seen from figure 6, the permanganate numbers agree well with WRIGHT's color grades for the darker sepias. It is especially noteworthy that all c^r combinations are higher than the corresponding c^d combinations in both, a reversal of the order in the yellow series.

The chief conclusions for the darker sepias aside from those already made by WRIGHT concern dominance relations of the lower alleles of the *C* series. It is seen that c^r has little or no dominance over c^a, that is, $c^r\ c^a$ has approximately one-half as much pigment as $c^r\ c^r$. Correcting the means for adsorbed impurities by subtracting 12 (the value for white), we get $c^r\ c^r = 119$, $c^r\ c^a = 65$. There is therefore 55 percent as much pigment in $c^r\ c^a$ as in $c^r\ c^r$. With the uncertainty of the means, it is impossible to tell whether or not there is a slight dominance of c^r, but it is quite likely that there is none. Similarly, there is 51 percent as much pigment in $c^d\ c^a$ as in $c^d\ c^d$. In WRIGHT's transformed grades the percentages are 55 percent and 70 percent respectively. There is little data for the c^k combinations. The observed percentage in $c^k\ c^a$, as compared with $c^k\ c^k$ (81 percent), is, however, practically the same as that in WRIGHT's transformed grades (82 percent). Thus c^d and c^r show approximate intermediacy of the heterozygote with c^a, while c^k shows considerable dominance over c^a. *C* in the sepia series has been found by WRIGHT's grades to be completely dominant in all combinations, and there is nothing in my data to alter this conclusion. WRIGHT suggests (1934) that "under the theory of control of rate" of a reaction in the series of processes between gene and character "by the limiting factor, there is exact intermediacy of the heterozygote (up to a certain level), then a range of increasing dominance of the higher allelomorph until at a certain level complete dominance is attained." This agrees well with the situation in the *C*-series acting on sepia pigment, except that c^k (in the sepia series a higher allele than c^d and c^r because of its partial dominance) homozygotes should be as high as *C*-. It is interesting to note that DUNN and EINSELE (1938) find a similar condition in mice, where the highest allele, *C*, is completely dominant in the black series and a lower allele, c^{ch}, shows no dominance (percent weight melanin in $C- = 7$, in $c^{ch}\ c^{ch} = 5$, and in $c^{ch}\ c^a = 2.5$.)

A striking contrast between the results for sepias and yellows is in the amount of difference between the values for the dominant *C* factor and for the lower alleles. In sepias, the corrected value in relation to 100 percent in *C E F P* is 82 percent in $c^k\ c^k$ *E F P*, and 72 percent in $c^d\ c^d$ *E F P*. In yellows the corresponding figures are 23 percent of *C e e* for $c^k\ c^k$ *e e* and 35 percent for $c^d\ c^d$ *e e* by the permanganate method, and 30 percent

and 38 percent by the colorimetric determinations which are undoubtedly more accurate. For the dominance effects of the lower alleles in yellows, only the colorimetric data seem worth considering. It must be remembered that both c^r and c^a are inactive in the yellow series. In these, $c^k\ c^r$ has 59 percent and $c^k\ c^a$ 44 percent as much color as $c^k\ c^k$. Similarly, $c^d\ c^r$ has 47 percent, and $c^d\ c^a$ 35 percent, as much as $c^d\ c^d$. *C* has been shown by WRIGHT to be completely dominant. These results suggest that in the yellow series as well as in the sepias, the gene product in relation to its substrate is in defect in the lower alleles, c^k and c^d, but in excess in *C*. There is no partially dominant allele here, but the wide gap between $c^{kd}\ c^{kd}$ and *C*- may account for this. The approximate identity of effect of c^k and c^d in effects on yellows, shown in WRIGHT'S grades, is also supported here. The uncorrected mean for $c^k\ c^k$ by the colorimetric method is $.940 \pm .055$, for $c^k\ c^d$ $1.031 \pm .151$, for $c^d\ c^d$ $1.150 \pm .182$, and all three means are within the limits of error of the mean of the combined population, $1.084 \pm .054$.

The results for the pale sepias are not as clear-cut as the others, considerable uncertainty resulting from lack of numbers in certain genotypes. The most striking feature is the great increase of *C* over $c^k\ c^k$, quite different from the darker sepias. However, the corrected value of 64 for *C*- depends on only one animal. The amount of pigment in $c^k\ c^k$ is 43 percent of that in *C*-, close to the ratio in yellows rather than to that in the darker sepias. The heterozygotes of c^k and c^d with c^a show intermediacy, as in the other series. The amount of pigment in $c^k\ c^a$ is 36 percent of that in $c^k\ c^k$, that in $c^d\ c^a$ is 20 percent of that in $c^d\ c^d$. The extremely high value of $c^d\ c^d$ is something which must be rechecked, as it is far out of proportion to the color.

The simplest interpretation for the *C* series of alleles, as far as they have been tested, seems to be that the dominance of *C* is brought about by the excess of its product somewhere in the chain of processes between gene and character, while the smaller product of the lower alleles is in defect in relation to the substrate in this same reaction. Also in some way the lower members of the albino series reduce yellow much more drastically than black, this effect being manifested not only in the difference in threshold (no yellow but much sepia in $c^r\ c^r$) but also in the relation of $c^{kd}\ c^{kd}$ to *C*-.

The E, e genes

The most striking difference observed between pigments produced with *E* and *e* is their solubility in NaOH. The sepia pigments, both in the hair and isolated, go into solution in cold alkali very slowly, and have never in my experimentation become anywhere near completely dissolved. EINSELE (1937) and DANIEL (1938) have been able to dissolve isolated mouse melanin by boiling in N/10 KOH for 3–5 hours, and the same is possible

for guinea-pig melanin, although the experiment had not been performed at the time of this work. The yellow melanin, on the other hand, dissolves completely and rapidly in cold dilute alkali. In fact, the use of soap had to be discontinued as its alkalinity dissolved out part of the pigment. This difference in solubility of melanins was found for the guinea-pig by DURHAM (1904). GORTNER (1911) describes alkali soluble and insoluble melanins from various sources, but makes no statement as to the relation of this solubility difference to color. SALLER and MAROSKE (1933) found this same difference in solubility in human hair, as did GOERNITZ (1923) in birds and DANNEEL (1936) in rabbits. Many authors, such as BOGART and IBSEN (1937), have maintained that this red pigment is present in the hair entirely in a dissolved state, but this cannot be true in the guinea-pig. In our experiments it has been possible to extract an appreciable amount of solid pigment from yellow hair by acid hydrolysis, and the amount of this pigment, as determined by the permanganate numbers, varies with the genotype in the same general way as that extracted in alkali. There seem to me to be two possible explanations for this greater solubility of the yellow pigment in alkali. The first is that the sepia pigment, although identical with the yellow chemically, is present in such large granules, so densely packed, that it cannot be attacked readily by the alkali. The other explanation of this phenomenon is a chemical difference between the two types of pigment. There is nothing in my experimentation which throws light on the nature of this difference. Working with mouse melanin, DUNN and EINSELE (1938) have found graded variations in the size of pigment granules from various genotypes in the black series, and DANIEL (1938) has found no significant differences in the chemical constitution of mouse melanin from various genotypes in a spectrophotometric study of the dissolved isolated pigment. However, it must be remembered that these papers deal entirely with the black and brown series, not with the difference between these and yellows, where the situation may be quite different. In the rabbit DANNEEL has found distinct differences in the histological arrangement of yellow and black pigment in the hair.

The F, f, P, p, and B, b genes

The effect of the *F*, *f* pair of genes on yellows was tested for two genotypes only, *C- e e f f* and $c^d\ c^d\ e\ e\ f\ f$. It is interesting to compare the reduction of yellow by combinations in the albino series in the presence of *f f* with that by the same combinations in the presence of *F-*. $c^d\ c^d$ has 37.5 percent as much pigment as *C-* with *F-*, but only 19 percent as much with *f f*. It may be noted that the reduction is even greater in $c^d\ c^a$, as $c^d\ c^a$ *F-* has considerable pigment, while $c^d\ c^a\ f\ f$ is pure white (except for occasional traces of cream on the nose, cf. WRIGHT 1927). In combination

with *E p p, f f* causes the removal of all pale sepia pigment. One genotype only, *E- C- f f p p* , was tested colorimetrically. The color and the ratio to *e e* c^d c^d were close to those of a grade 4 cream, the ratio being .469 ± .038.

The effect of *p p* aside from that in conjunction with *f f* is to make sepia paler, but whether this is entirely due to a decrease in quantity of the same pigment, or a change in the type of pigment, can not be stated here.

Whatever change *p p* produces in sepia pigment, the small amount of data collected here indicate that it has the same effect on browns; that is, *C- E- p p B-* has 49 percent as much pigment as *C- E- P- B-*, and *C- E- p p b b* has 45 percent as much as *C- E- P- b b*.

SUMMARY

1. A method has been developed for colorimetric comparison of alkaline solutions of yellow pigment of guinea-pig hair. Tests have been made of all available genotypes.

2. The percent of the total weight of the hair formed by the melanin has been determined in two of the darker sepia genotypes by the EINSELE technique.

3. A method for determining the melanin content of sepias, pale sepias, and, to a limited extent, yellows, has been developed by a modification of the EINSELE technique, in which the isolated pigment is oxidized with potassium permanganate. Tests of all available genotypes have been made with this method.

4. The dominance relations of *C*, c^k, c^d, c^r, c^a indicate that in sepias, pale sepias, and yellows, the homozygotes of the lower active alleles produce approximately twice as much pigment as their heterozygotes with the inactive gene or genes, while the highest allele appears to be completely dominant. This suggests that in some reaction between gene and character the gene product is in defect in relation to the substrate with the lower alleles and in excess with the highest member of the albino series.

5. The lower alleles of the *C* series reduce the amount of pigment much more drastically in yellows and pale sepias than in darker sepias.

6. Measurements of the effects of the *F, f, P, p*, and *B, b* series are given, with some suggestions as to the nature of the action of these genes.

LITERATURE CITED

BACH, A., 1908 Zur Kenntnis der in Tyrosinase tätigen Peroxydase. Ber. deuts. chem. Ges., **41**: 216–220.

BERTRAND, M. G., 1896 Sur une nouvelle oxidase ou ferment oxydent d'origine vegetable. C. R. Acad. Sci., **122**: 1215–1217.

BLOCH, B., 1917 Chemische Untersuchungen über das spezifische pigmentbildende Ferment der Haut, die Dopaoxydase. Z. Phys. Chem., **98**: 226–254.

BOGART, R., and IBSEN, H. L., 1937 The relation of hair and skin pigmentation to color inheritance in cattle, with some notes on guinea-pig hair pigmentation. J. Genet. **35**: 31–60.

DANIEL, J., 1938 Studies of multiple allelomorphic series in the house mouse. III. A spectrophotometric study of mouse melanin. J. Genet. **36**: 139–143.

DANNEEL, R., 1936 Die Färbung unserer Kaninchenrassen und ihre histogenetischen Grundlagen. Z.i.A.V. **71**: 231–264.

DUNN, L. C., and EINSELE, W., 1938 Studies of multiple allelomorphic series in the house mouse. IV. Quantitative comparisons of melanins from members of the albino series. J. Genet. **36**: 145–152.

DURHAM, F. R. 1904 On the presence of tyrosinase in the skin of some pigmented vertebrates. Proc. Roy. Soc. London, **74**: 310–313.

EINSELE, W., 1937 Studies of multiple allelomorphic series in the house mouse. II. Methods for quantitative estimation of melanin. J. Genet. **34**: 1–18.

FÜRTH, O., Melanine, Lipochrome, Farbstoffe der Wirbellosen. In: Oppenheimer's Handb. d. Biochem., **1**: 944–960.

GOERNITZ, K., 1923 Versuch einer Klassifikation der Häufigsten Federfarbungen. J. Orn., 71.

GORTNER, R. A., 1911 On melanin. Biochem. Bull., **1**: 207–215.

1912 On two different types of melanin. Proc. Soc. Exp. Biol. Med. **9**: 3–4.

RAPER, H. S., 1928 The aerobic oxidases. Physiol. Rev. **8**: 245–282.

1932 Tyrosinase. Ergebn. Enzymf. **1**: 270–279.

RUSSELL, W. L., 1939 Physiological genetics of guinea-pig coat color investigated by means of the dopa-reaction. In press.

SALIER, K., and MAROSKE, F., 1933 Chemische und genetische Untersuchungen an menschlichen Pigmenten, speziell demjenigen des Haares. Z. Konst Lehre., **17**(3): 279–317.

SAMUELY, F., and STRAUSS, E., 1922 Tierische Pigmente und Farbstoffe. In: Aberhalden's Handb. biol. Arb Meth., Abt. 1, Teil 8, 731–790.

WRIGHT, SEWALL, 1916 An intensive study of the inheritance of color and other coat characters in guinea-pigs with especial reference to graded variation. Carnegie Inst. Wash. Publ., **241**: Part II, 59–121.

1917 Color inheritance in mammals. J. Hered. **8**: 224–235.

1925 The factors of the albino series of guinea-pigs and their effects on black and yellow pigmentation. Genetics **10**: 223–260.

1927 The effects in combination of the major color factors of the guinea-pig. Genetics **12**: 530–569.

1934 Physiological and evolutionary theories of dominance. Amer. Nat. **68**: 24–53.

PLASTID VARIEGATION AND CONCURRENT ANTHOCYANIN VARIEGATION IN SALPIGLOSSIS[1]

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A VARIEGATED strain of Salpiglossis has now been carried in the senior author's cultures for eight years. This strain is unique in that the variegation affects both the leaves and the flowers as reported in a preliminary note (DALE and REES-LEONARD 1935). The variegation pattern, typical of many chlorophyll variegations, is shown in the leaves of an adult plant (figure 1). The leaves are marked by sharply defined spots of diverse sizes and shapes which vary in color from light green to white. It should be noted that white branches have not been found on the variegated plants. In seedlings (figure 2), the variegation shows some rather striking differences from the adult type. The pattern is coarser with relatively greater amounts of white or pale tissue. This increase in chlorophyll deficient tissue often results in crumpling or asymmetry of the leaves. In variegated seedlings, also, the bases of the leaf blades frequently exhibit white or pale borders. For the sake of comparison, a normal seedling (figure 4) is illustrated. The seedlings were of the same age, growth being retarded in variegated plants.

The flower color in anthocyanin types of Salpiglossis is due to a combination of anthocyanin with yellow plastids. Absence of anthocyanin gives yellow flowers. Both the yellow and anthocyanin colors show variegation. Naturally, the color of the pale areas in variegated flowers depends upon the ground color of the flower, yellow flowers having very light yellow spots and anthocyanin colors showing an apparent modification of the anthocyanin, the nature of which is discussed later. A variegated yellow flower is illustrated (figure 3). Some regularity of pattern may result from the elongation of the pale sectors in a direction acutely pinnate to the axis of each lobe. As in the leaves, the spots are always sharply defined. Marked variation occurs in the amount of variegation in flowers of a single plant. In particular, the first flower or flowers may be slightly, or, so far as the

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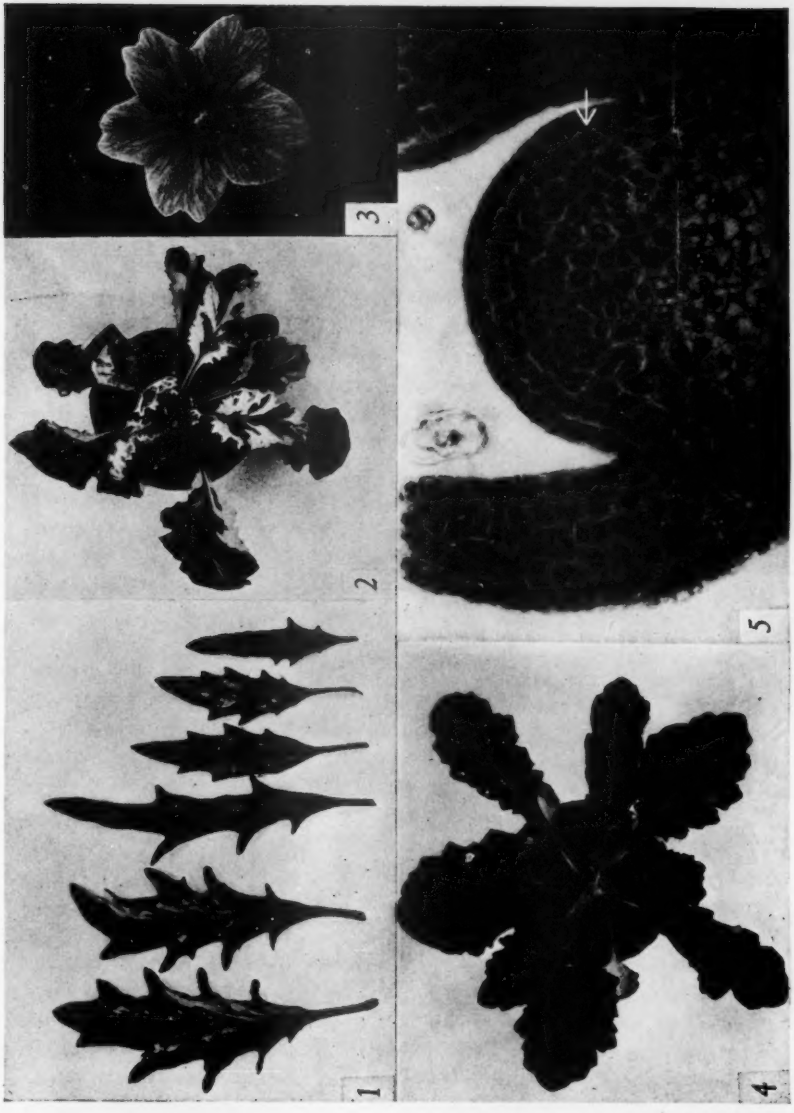
EXPLANATION OF FIGURES 1–5

FIGURES 1–5.—Fig. 1. Leaves of adult variegated Salpiglossis plant.—Fig. 2. Variegated seedling.—Fig. 3. Variegated flower.—Fig. 4. Normal seedling.—Fig. 5. Longitudinal section of stem tip × 500. Note periclinally dividing cell of outermost periblem layer at right and nearby in same layer a cell which has divided periclinally. Figs. 1–4 natural size. Photographs 1–5 reduced approximately one-fourth in reproduction.



FIGURES 1–5. (See opposite page for description.)

eye can see, not at all *variegated* but such flowers usually show variegation when examined with a hand lens of medium power.

HISTOLOGY OF THE VARIEGATION

Variegated leaves

For the study of the cell structure of variegated leaves fresh material proved much superior to tissue fixed in formalin acetic alcohol, FLEMMING'S medium, or ERLICKI'S as modified by ZIRKLE and ANDERSON. The general effect of all the fixatives was the same; the colorless cells tended to go to pieces leaving only collapsed and irregular fragments. For the preparation of fresh material, sections were cut using a hand microtome, the sections were immersed in 95 percent alcohol for a half-minute or a minute and then mounted in glycerine. The mesophyll of the normal leaf (figure 6) consists of a single layer of palisade cells and three or sometimes four cell layers of spongy tissue. Variegation may affect any or all of these layers. The variegated areas invariably show a sharp transition from green to colorless cells. All sections were cut parallel to the secondary veins of the blade. The aim was, as far as possible, to show the developmental succession of the cells. Illustrations of typical arrangements of green and colorless cells in the variegated areas are shown (figures 7–10) in which the green cells are stippled: the colorless cells are shown in outline only. We have been quite unable to demonstrate plastids in the colorless cells, either in fresh or in fixed and stained material. Any one of the mesophyll layers may show colorless cells, or various combinations of green and colorless layers may occur. The occurrence of colorless areas limited to each of the mesophyll layers shows that change may take place anywhere in the mesophyll.

KÜSTER (1927) described the changes in cell structure occurring in variegated leaves of several different species and pointed out that tissue differentiation in the pale parts may be incomplete and that overgrowth of the palisade cells often results when the palisade is underlain by colorless mesophyll. Since the hereditary nature of KÜSTER'S variegations is not stated and probably differed in his various examples, the modifications found in variegated Salpiglossis leaves will be described in some detail.

It is characteristic of colorless mesophyll cells in Salpiglossis that they fail to differentiate and this may lead to a loss of dorsi-ventrality in the leaf (figures 8, 10, 11). Colorless palisade cells (figures 7, 8, 10) are much smaller than normal palisade (figure 6), whereas green palisade adjacent to *colorless* shows characteristic enlargement of the cells (figures 9, 10). Furthermore, a comparison of normal and colorless palisade tissue (figures 6, 7, 8) suggests that some reduction in the amount of cell division has occurred in the colorless areas. The spongy mesophyll of variegated regions shows changes similar, on the whole, to those noted for the palisade. But

green spongy cells bordered by a colorless layer undergo a marked change in shape as well as in size. For example, the green cells (figure 8 layers 2 and 4; figure 9 layer 3; and figure 10 layer 4) have lost the irregular shape characteristic of normal spongy cells and have become palisade-like. The green cells abutting on *colorless* would probably encounter less resistance to elongation than the corresponding cells of a normal leaf and the changes described might well result from a difference in pressure.

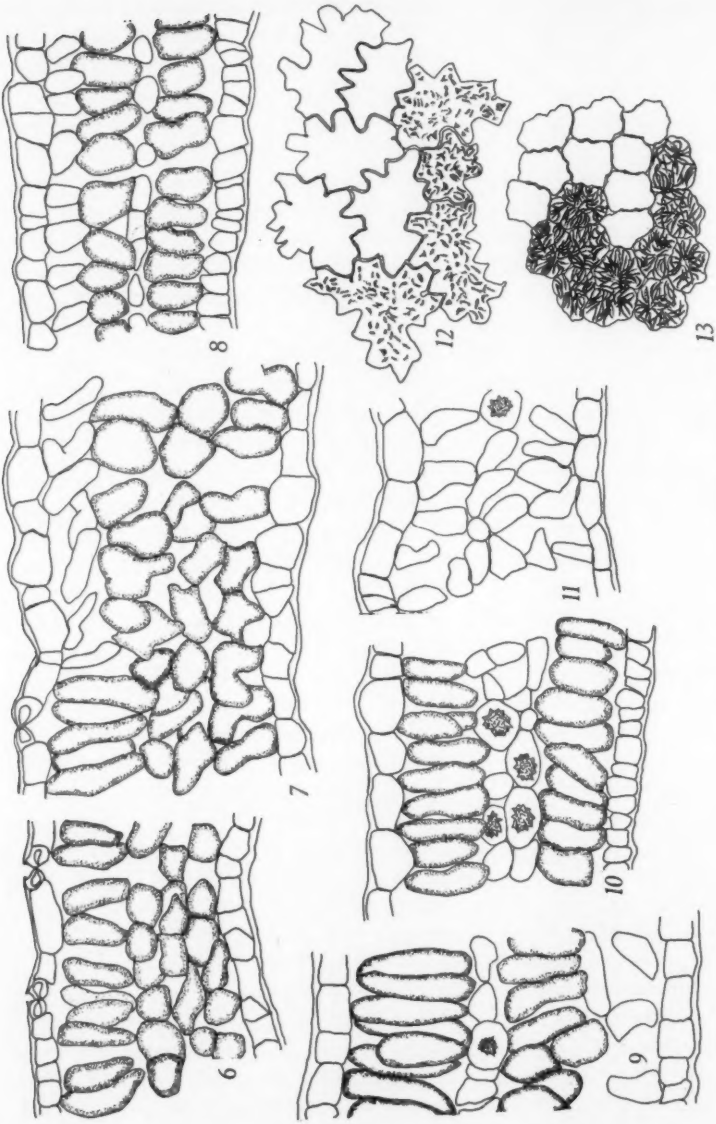
An unexpected feature of variegated Salpiglossis leaves is that chloroplasts were found in the epidermis of the white spots, both in the ordinary epidermal cells and the guard cells. This agrees with RISCHKOW'S observation (1927) that chlorophyll may be present in the epidermis of variegated leaves and absent in the mesophyll. It may be observed, further, that the occurrence of chloroplasts in leaf epidermis (LINSBAUER 1930) is by no means exceptional.

Variegated flowers

Both fixed and stained preparations and sections from fresh material proved unsatisfactory for the study of the structure of variegated flowers, but the variegation was readily observed in fresh upper and lower floral epidermis mounted in water (figures 12, 13). Yellow plastids (chromoplasts) are present in the epidermal and mesophyll cells of the corolla. They are very abundant in the upper epidermis, somewhat less numerous in the lower epidermis and sparingly present in the mesophyll. The plastids are typically sickle-shaped but rounded and triangular forms occur. Microscopic study of the epidermis of variegated flowers shows that variegation involves the plastids. The pale cells usually show no indication of plastids (figures 12, 13) but abnormal rounded plastids sometimes occur. In either case, the borders of the pale spots are sharply defined. The abnormal plastids are believed to be degenerate. It is not clear whether the change from normal to pale cells affects the amount or character of the anthocyanin produced by a cell. The color, however, is modified. For example, variegation in dark red flowers gives purple spots. Since variegation occurs in both yellow and anthocyanin types, it seems clear that the primary effect is upon the plastids and that the anthocyanin is secondarily involved.

DIRECTION OF CHANGE IN THE VARIEGATION

The marking of the variegated leaves indicates that the direction of change is from *green* to *colorless*. Seedling leaves, however, sometimes show single green spots on relatively large areas of colorless tissue and these suggest that the reverse change also occurs. But some green spots on *colorless* would be expected simply as a result of the chance arrangement of the pale spots in a strongly variegated leaf in which the direction of change was from *green* to *colorless*. Probably this is the explanation of the occa-



FIGURES 6–13. (See opposite page for description.)

sional spots referred to. There is, however, a possibility that the green spots on white may have an epidermal origin, since the marginal mesophyll of leaves may sometimes be derived from dermatogen (JONES 1934) and, as has been seen, the epidermis of the variegated Salpiglossis leaves contains chloroplasts.

It will be recalled that white branches were not found on variegated Salpiglossis plants. This is difficult to reconcile with the view that the variegation is the result of change from *green* to *colorless*. But the indications of reduction in the amount of cell division in colorless as compared with normal palisade tissue suggested that a histological study of the leaves and growing points of the stems might shed some light on the problem. Using a 43× objective and a 7.5× ocular of which the micrometer scale length was equal to 185μ at stage level, counts were made in the palisade tissue of the leaves to determine the ratio of the number of green to colorless cells. Two hundred and sixty counts of colorless palisade averaged 6.06 cells for the length of the micrometer field while the same number of counts of green palisade averaged 10.2 cells. 21 leaves were used, counts of both *green* and *colorless* being obtained in each case. The counts were made in plants of an inbred variegated strain and only leaves with blades 1–1.5 inches in length were used.

The ratio of the number of colorless to green palisade cells as seen in cross sections of the leaves is 1:1.7. If this ratio is used as an index of the mean rate of multiplication of these cells, then, other factors being equal, a meristematic green cell would produce seventeen cell generations while a similar but colorless cell was producing ten. In other words, 128 times as many green as colorless cells would result during the period.

The consequences of such lag in the growth of the colorless cells would be influenced by the character of the growing point. In Salpiglossis the growing point of the shoot shows a distinct sheath-like arrangement of only the dermatogen and the outermost layer of the periblem, the deeper lying cells being irregularly arranged (figure 5). Furthermore, as shown in the figure, periclinal divisions may occur even in the outer periblem layer. Thus, if a comparable difference in the rate of multiplication of green and colorless cells as shown for the palisade of the leaves obtains also in the

EXPLANATION OF FIGURES 6–13

FIGURES 6–13.—Fig. 6. Cross section of normal Salpiglossis leaf.—Fig. 7. Section of variegated leaf showing normal (green) and colorless palisade, spongy cells *normal*.—Fig. 8. Palisade and second spongy layer *colorless*, first and third spongy layers *normal*.—Fig. 9. Palisade and second spongy layer *normal*, first and third spongy layers *colorless*.—Fig. 10. Palisade and third spongy layer *normal*, first and second spongy layers *colorless*.—Fig. 11. All mesophyll cells *colorless*.—Fig. 12. Lower epidermis from variegated flower showing normal cells (with chromoplasts and pale cells (without chromoplasts).—Fig. 13. Upper epidermis from variegated flower showing normal and pale cells. All figures drawn with camera lucida at 930×. Reduced two-fifths in reproduction.

shoots, there would be a tendency for colorless cells to be supplanted by green cells at the growing points. It seems very probable that supplanting of *colorless* by *green* accounts for the failure of the variegated plants to produce white branches.

In variegated Salpiglossis flowers the direction of change is apparently from *normal* to *pale*. Extreme variegation produces in addition to pale spots on a dark background, frequent examples of dark (normal) spots on pale. These latter might be interpreted as "reverted" spots but study shows that they are markedly irregular in form and also that spots of one to thirty cells in size are conspicuously absent from the "reversions." Small spots would be expected if reversion occurred with observable frequency. Accordingly, we conclude that the "reverted" spots are formed by the confluence of the pale areas.

CHROMOSOME NUMBER

Chromosome counts of pollen mother cells showed that variegated plants have the normal ($n = 22$) chromosome number.

BEHAVIOR OF VARIEGATED

A total of 587 variegated-leaved plants all produced variegated flowers. As was to be expected, normal leaves and flowers occurred on variegated plants. A check of 1036 leaves from 12 plants of an inbred variegated strain showed 961 *variegated* : 75 *normal*. Since no attempt was made to measure the frequency or size of the spots on individual leaves, these figures do not express any precise relationship between *variegated* and *normal*. They are useful, however, for descriptive purposes. The data on the production of normal flowers by variegated plants indicate that the normal flowers tend to occur early in the flowering period. The percentage of normal flowers borne on 27 plants of an inbred variegated strain was as follows: 21 percent on June 21; 3 percent on July 7; all *variegated* on August 10; and all *variegated* on September 6. Observations were made on 398 flowers.

Our records show only one variegated-leaved plant which failed to produce variegated flowers. This plant died after producing only three flowers and was, therefore, not critically checked. One other case should be mentioned in this connection. A plant from F_2 of the cross *variegated* × *normal* produced 39 progeny in the first inbred generation. 38 of these plants were *variegated* and showed marked variation in the amount of spotting. The remaining plant, checked repeatedly during a period of about five months, was not observed to be *variegated*. But an inbred progeny of 28 plants of this latter individual were all *variegated* although the intensity of the variegation was reduced in comparison with ordinary variegated strains. Thus the exceptional plant must have been homozygous *variegated*, the amount of variegation being reduced, presumably by modifying factors.

To test the possibility that the variegation might be due to disease, tissue of strongly variegated plants was macerated in a little water and inoculated into normal plants after wounding of the leaves. 67 six-weeks-old seedlings were so treated. In addition, 97 seedlings four weeks old were sprayed with a solution containing macerated tissue. After three months, no indications of variegation having appeared in the treated plants, the experiment was discontinued.

Seeds of variegated Salpiglossis give a small percentage of pale seedlings and these die soon after the cotyledons unfold. Seeds homozygous for variegation gave 913 *normal* : 24 pale seedlings or 2.6 percent of *pale*. Seeds of heterozygous *variegated* gave 1023 *normal* : 3 *pale* or 0.3 percent of *pale*. Some anthocyanin color may be present in the pale seedlings.

The time of the first appearance of variegation is somewhat variable. In 61 seedlings the first stage to show *variegated* (or pale) was as follows:

Cotyledons *variegated* (or pale)	7 seedlings
First leaf *variegated*	15 seedlings
Second leaf *variegated*	12 seedlings
Third leaf *variegated*	18 seedlings
Fourth leaf *variegated*	6 seedlings
Fifth leaf *variegated*	3 seedlings

When *variegated* was crossed with *normal*, the F_1 was *normal*. F_2 and backcross progenies were obtained each year from 1932 to 1935. For reasons which will appear later the data of 1932–33 have been separated from the data of 1934–35. Table 1 gives the F_2 progenies for 1932–33.

TABLE 1

F_2 segregation for variegated 1932–33.

CULTURE NUMBER	NORMAL	VARIEGATED	EXPECTED	STANDARD ERROR
731	28	9		
939	79	16		
940	62	21		
	Total 169	46	161:54	6.0

The data indicate simple recessive behavior of *variegated*, although culture 939 shows rather wide departure from expectation. Table 2 gives the backcross progenies.

Cultures 732 and 733 were grown in 1932, the remainder in 1933. The fact that all the cultures grown in 1933 show an excess of *normal* and the

TABLE 2

Segregation for variegated in backcrosses 1932–33.

CULTURE NUMBER	NORMAL	VARIEGATED	EXPECTED	STANDARD ERROR
732	29	34		
733	21	27		
944	34	19		
945	45	25		
948	64	59		
949	73	46		
950	25	20		
951	44	29		
952	38	25		
	Total 373	284	329:328	12.7

rather wide departures from expectation found in cultures 944, 945, 949, and 951 indicate, if variegation is in fact inherited as a simple recessive character, that disturbing factors were present. Disturbance of expected ratios might be due to genetic modifiers, to factors affecting gametic or zygotic viability, or to environmental factors. Inasmuch as normal segregation was later (table 4) found to occur in backcrosses regardless of whether the male or female parent was heterozygous, it appears that the gametes did not show differential viability with respect to *variegated*. Moreover, a pollen count of 1468 for *variegated* showed 96.4 percent of normal grains. There was likewise no evidence that deficiency for *variegated* was due to a lower viability of variegated zygotes since seed germination in a variegated stock was 93 percent (186 out of 200). It remains to consider the possibility that the disturbed ratios were due to genetic modifiers or to environmental factors. That genetic modifiers do occur is shown by the fact that progenies segregating for *variegated* exhibit such a wide range of variegation, that, to obtain accurate counts, it is necessary to check the plants at least twice, allowing a suitable interval between counts. Otherwise, weakly variegated plants may be missed. Furthermore, types showing reduced variegation breed true.

It was found that seedlings of variegated Salpiglossis had a tendency to damp off in the seed pans. Since it seemed likely that genetic modifiers and damping-off might both be involved in the production of the aberrant ratios referred to, in the subsequent crosses an effort was made to eliminate both types of disturbing factors. Repeated backcrossing of heterozygous *variegated* to *variegated* was used to get rid of modifiers. Damping-off was guarded against by using only sterilized soil in the seed pans and by special care in watering. The further data obtained are given in tables 3 and 4.

TABLE 3

Segregation for variegated in F_2 1934–35.

CULTURE NUMBER	NORMAL	VARIEGATED	EXPECTED	STANDARD ERROR
1023	10	7		
1024	19	6		
1027	62	24		
1028	76	22		
1044	43	15		
1044.2	64	20		
1065	12	3		
1066	27	6		
1067	24	7		
1068	28	13		
1069	20	7		
1070	36	6		
1071	27	4		
1072	36	16		
1073	92	28		
	Total 576	184	570:190	11.8

Backcross data for the same period are given below.

TABLE 4

Segregation for variegated in backcrosses 1934–35.

CULTURE NUMBER	NORMAL	VARIEGATED	EXPECTED	STANDARD ERROR
1029	33	37		
1030	48	52		
1031	56	43		
1032	43	45		
1074	49	52		
1075	56	63		
1076	61	65		
1077	64	47		
1078	24	24		
1079	68	94		
Total	502	522	512:512	16.0

The data of tables 3 and 4 show beyond a reasonable doubt the simple recessive inheritance of *variegated* and they indicate also a differential selection by damping-off.

The possibility that the leaf and flower variegations are due to distinct but closely linked genes must be recognized. But except for the case already mentioned, of a variegated-leaved plant which died after producing three normal flowers, we have found no instance of separate leaf and flower

variegations in our stocks. And it is significant that 66 F_2 and 134 back-cross progeny all of which bore variegated leaves likewise produced variegated flowers. Accordingly, we conclude that the variegations represent different though homologous effects of the same gene.

DISCUSSION

It is well-known that the same plastids may be in turn colorless, green, and yellow at different periods in their history. On the basis of such transformations, there is a general belief that the different types of plastids, leucoplasts, chloroplasts, and chromoplasts are homologous structures. The occurrence of a gene having similar effects upon the chloroplasts and chromoplasts supports the idea of their homology. There would seem to be a possibility that the gene for *variegated* might mutate to a type affecting only one kind of plastid, but, as already observed, we have found no case of this kind. It would perhaps be possible to make synthetically a type with variegated leaves which would show little or no variegation in the flowers. Pale-flowered strains of Salpiglossis occur, due to a factor or factors which inhibit the development of normal plastids in the limb of the corolla. The combination of the inhibited-plastid type with *variegated* would be expected to produce a plant in which the variegation, although genetically present, would not manifest itself in the flowers.

The extensive literature of chlorophyll variegations has been summarized by DEHAAN (1933) in his monograph on chlorophyll deficiences. Either a mutable gene or a pattern gene producing an unstable phenotype could explain the behavior of the variegation in Salpiglossis. ROELOFS's term, "phenotypically eversporting" (1937), is to be preferred as a name for variegations of the unstable pattern type. That the Salpiglossis variegation is not due to plastid mutation in the sense of IMAI (exomutation, 1934) is indicated first, by the fact that sections of variegated leaves have shown only fully green or colorless cells, there being no evidence of transitional stages. Second, the fact that in variegated flowers it is possible to examine all the epidermal cells immediately surrounding "mutated," that is, pale epidermal spots and that here, too, the change from normal to pale cells is abrupt and has clearly occurred in one cell generation, appears to rule out the plastid mutation hypothesis.[2] Third, a plastid-transmitted change could hardly account for the absence of plastids in the pale cells of the variegated plants.

The evidence as to the genetic character of the variegated tissues in Salpiglossis is, unfortunately, conflicting. Failure of these plants to segregate out white or green branches or green seed progeny points to the operation of a gene with an unstable phenotype, whereas the production of a

[2] Shown especially well in the smaller spots consisting of 1–5 cells.

small percentage of white seedlings in the progeny of variegated suggests that a mutable gene is involved. The fact that a variegation of the phenotypically unstable type in Lunaria produced variegated seedlings (CORRENS 1909) raises the question whether such factors may not sometimes produce white seedlings. In the absence of critical evidence concerning the genetic nature of the two types of tissue produced in variegated Salpiglossis plants, the question of mutable gene versus unstable phenotype in this variety should, for the present, be left open.

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SUMMARY

1. A variegated strain of Salpiglossis shows variegation in both the leaves and the flowers.

2. The variegation is determined by a recessive factor which inhibits the development of chloroplasts in the leaves and chromoplasts in the flowers.

3. Yellow and anthocyanin flower types both show the variegation.

4. There is a secondary effect of the plastid modification upon anthocyanin pigmentation.

5. Variegated leaves show evidence of reduction in the amount of cell division in colorless as compared with normal green palisade cells.

6. The direction of change in the variegation is apparently from *normal* to *pale* in both the leaves and the flowers.

LITERATURE CITED

CORRENS, C., 1909 Vererbungsversuche mit blass (gelb) grünen und buntblättrigen Sippen bei *Mirabilis jalapa*, *Urtica pilulifera* und *Lunaria annua*. Z. i. A. V. **1**: 291–329.

DALE, E. E., and REES-LEONARD, OLIVE L., 1935 A variegation in Salpiglossis (Preliminary report before Section G of the A. A. A. S., St. Louis, Mo.).

DEHAAN, H., 1933 Inheritance of chlorophyll deficiencies. Bibliogr. Genet. **10**: 357–416.

IMAI, Y., 1934 An apparently simple inheritance of variegation in *Polygonum orientale*. J. Genet. **29**: 147–151.

JONES, W. NEILSON, 1934 Plant chimeras and graft hybrids. London.

KÜSTER, E., 1927 Anatomie des panaschierten Blattes. Linsbauer's Handb. Pflanzenanatomie, Abt. II, **7**: 2. Berlin.

LINSBAUER, K., 1930 Die Epidermis. Linsbauer's Handb. Pflanzenanatomie, Abt. I, **4**: 2. Berlin.

RISCHKOW, V., 1927 Die Verbreitung des Chlorophylls und der Peroxydasegehalt der Epidermis bei buntblättrigen Pflanzen. Biol. Zbl. **47**: 501–512.

ROELOFS, E. T., 1937 Phenotypical and genotypical eversporting varieties. Genetica **19**: 466–536.

A ZYGOTIC LETHAL IN CHROMOSOME 1 OF MAIZE AND ITS LINKAGE WITH NEIGHBORING GENES[1]

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DISTORTED F_2 RATIOS OF CHARACTERS LINKED WITH A ZYGOTIC LETHAL

A BOLIVIAN maize with mosaic red pericarp and cob, here designated by the symbol *M-M*,[2] crossed with a local inbred strain of maize having white pericarp and cob, *W-W*, produced in F_1 21 *M-M* and 28 *W-W* plants, not far from the 1:1 relation expected on the assumption that the *M-M* parent was heterozygous for pericarp and cob color, *M-M/W-W*. In F_2 and segregating F_3 cultures, however, there were 130 *M-M* and 64 *W-W* plants obviously a 2:1 instead of the 3:1 relation expected. Later cultures increased these records to 747 *M-M* and 382 *W-W* plants, a deviation of only 6 ± 10.5 from a 2:1 ratio. Of 18 *M-M* plants tested, all were heterozygous for pericarp color, though one-third were expected to be homozygous. The odds against the chance occurrence of this deviation of 6 ± 1.3 are over 500:1. This suggested that the 2:1 ratio might be brought about by the elimination of homozygous *M-M* plants.

To test this possibility, *M-M* plants were crossed with an inbred *W-R* strain. The F_1 cultures consisted of 195 *M-R* and 183 *W-R* plants (really *M-M/W-R* and *W-W/W-R*). In F_2 and later segregating cultures from *M-R* parents, there were 1238 plants with mosaic and 617 with white pericarp, again a 2:1 relation. The 617 were *W-R* as expected. Of the 1238 plants with mosaic pericarp, 1217 were *M-R* and 21 *M-M*, that is, 1217 were heterozygous *M-M/W-R* and only 21 homozygous *M-M/M-M*. The fortunate circumstance that, in crosses involving *M-M* with *W-R* all heterozygous plants are *M-R* and homozygous ones *M-M*, makes it possible to determine hetero- and homozygosity by mere inspection of the ears, thus avoiding the necessity of growing innumerable progenies. The results

[1] Paper No. 224, Department of Plant Breeding, Cornell University, Ithaca, New York. Many of the field records used in this paper were made by DR. G. A. LEBEDEFF.

[2] The gene *P* for pericarp color has numerous alleles (ANDERSON 1924; EMERSON, BEADLE, and FRASER 1935). Thus, the symbols P^{rr}, P^{wr}, P^{vv}, etc., indicate red pericarp and red cob, white (colorless) pericarp and white cob, variegated pericarp and variegated cob, respectively. For convenience in this paper, the locus of these genes is indicated by *P* without superscripts and the several alleles by the superscripts alone, the first letter for pericarp and the second for cob color as follows: *R-R* red pericarp and cob, *M-M* mosaic pericarp and cob, *V-V* variegated pericarp and cob, *W-W* white pericarp and cob, *W-R* white pericarp and red cob. The symbols *M-R* or *V-R* are used to indicate mosaic or variegated pericarp associated with red cob because of the dominance of mosaic or variegated over white pericarp and of self red over variegated or mosaic cob in the heterozygotes *M-M/W-R* or *V-V/W-R*.

recorded here indicate clearly that the 2:1 relation of mosaic to white is due to the almost complete elimination of the expected 25 percent of homozygous *M-M* plants. This result is ascribed to a recessive zygotic lethal, *zl*, closely linked with *P*. This hypothesis is supported by the observation that the ears of selfed plants heterozygous for *zl* show partial sterility. Theoretically there should be 25 percent of missing kernels, but it is usually impossible to find that many vacancies. Reciprocal crosses of heterozygous *zl* plants with homozygous normals give a full set of kernels, thus showing that no gametic lethal is involved.

The inbred *W-R* strain used in the crosses noted above was segregating for a then unknown recessive male-sterile gene, now known as ms_{17}. Of F_2 and later cultures in which ms^3 was involved there were 673 normal and 341 *ms* plants, clearly a 2:1 relation. Moreover, of the 673 normal plants, 641 were *M-R*, six *M-M* and 26 *W-R*, while of the *ms* plants 329 were *W-R* and 12 *M-R*. Evidently, *ms* is closely linked with *P* and with *zl*.

It has long been known that tassel-seed 2, ts_2, is closely linked with *P*. It was assumed, therefore, that it must be linked with *ms* and with *zl*. Mosaic-pericarp plants of the cultures recorded above were, therefore, crossed with *W-R* plants carrying *ts*.[3] Parental *M-R* plants gave in F_2 and later generations 376 normal and 176 *ts* plants. Of the normals, six were *M-M*, 357 *M-R*, and 13 *W-R*; while, of the *ts* plants 10 were *M-R* and 166 *W-R*. Here *zl*, again, as expected, eliminated most of the homozygous *M-M* plants resulting thereby not only in a 2:1 ratio of colored to white pericarp but also in a 2:1 ratio of normal to *ts* plants.

The data presented above show that *zl* is linked with the dominant mosaic red pericarp and the normal allele of *zl* with the recessive white pericarp. The convenient allelic series of pericarp-color genes makes it easily possible to arrange a cross in which *zl* is linked with a recessive pericarp color and its dominant normal allele with a dominant color, and this without having to use a previous crossover. Self-red pericarp and cob, *R-R*, is dominant to *M-M* just as *M-M* is to *W-W*. Maize with *M-M zl* was crossed with *R-R ts* and resulted in normal *R-R* F_1 plants. There resulted in F_2 123 normal and 49 *ts* plants. All the *ts* and all but one of the normal plants were *R-R*; the one exception was *M-M*. The ratio of *R-R* to *M-M* was 171:1. Later records increased these numbers to 705:7. Some of the F_1 *R-R* plants involved here were also crossed with normal *W-W* plants and produced in the test-cross generation 93 *R-R* and 95 *M-M* plants (*R-R/W-W* and *M-M/W-W*, respectively). Obviously *R-R/M-M* F_1 can give *M-M* plants in F_2 only when *zl* is lost from at least one *M-M* gamete (*M-M zl/M-M+*).

[3] Since ms_{17} and ts_2 are the only male-sterile and tassel-seed genes referred to in this paper, they are here designated by the symbols *ms* and *ts* without subscripts.

INTENSITY OF LINKAGE BETWEEN *zl*, *P*, ms_{17}, AND ts_2

It is evident from the foregoing that in chromosome 1 of maize there is a group of four closely linked genes, *P*, *ms*, *ts*, and *zl*. No unusual difficulty is experienced in determining the percent of recombination between *P* and *ts* or *P* and *ms*. The following summary (table 1) is taken from EMERSON, BEADLE, and FRASER 1935:

TABLE 1

X Y	LINKAGE PHASE	X Y	X y	x Y	x y	TOTAL	RECOMBINATIONS	
							NUMBER	PERCENT
$Ms_{17}P$	*C B*	1307	36	45	1318	2706	81	3.0
P Ts_2	*C B*	1558	19	21	1510	3108	40	
	R B	0	94	92	2	188	2	
						3296	42	1.3

These results indicate that *ts* is nearer *P* than *ms* is and that the two are either approximately 1.7 or approximately 4.3 units apart. It is impossible, however, to measure this distance by ordinary genetic tests, without excessive effort. Male-sterile 17 almost never produces viable pollen and most lines of tassel-seed 2 are almost entirely pistillate flowered. It is, therefore, in most instances, impossible to identify the double recessive. The occurrence of a double recessive, however, doubtless by crossing over, has been demonstrated. Crosses of male-sterile with tassel-seed (one or other parent heterozygous normal) gave normals in F_1 and, in F_2 and later segregating generations, normal, male-sterile, and tassel-seed plants in the following numbers, respectively: 428, 196, 161, approximately a 2:1:1 relation. One of these male-steriles with variegated pericarp and red cob, *V-V ms/W-R ms*, was crossed by a normal *W-R*, resulting in normal F_1's *V-V ms/W-R+* and *W-R ms/W-R+*. One of these *V-R* normals on being selfed gave in F_2 15 *V-R* normal, four *W-R* normal, six *V-V ts*, and no *ms*. Fortunately, this same *V-R* normal parent had been crossed on both *W-R ts* and *W-R ms*. The first of these test crosses resulted in nine *W-R* normal and 16 *V-R ts* and the second in 22 *W-R* normal and 12 *V-R ms*. Obviously, the original male-sterile plant that had been crossed by a *W-R* normal carried *ts* as well as *ms*. The F_1 was *V-V ms ts/W-R++* and the F_2 *ts* plants were really double recessives, *ms ts*. This situation could have resulted only by a crossover between *ms* and *ts*.

Since homozygous *zl* plants never appear, backcross data cannot be obtained and linkage intensity for *P* and *zl* must, therefore, be calculated from F_2 progenies. As shown earlier in this account, plants heterozygous

for *M-M*, *W-R* and *zl* (*M-M zl/W-R+*) give in F_2 a 2:1 ratio of mosaic to white pericarp, the former associated almost always with red cob as in the F_1 parent. The few mosaic-cob plants observed must have resulted from crossovers whereby *zl* was lost from one *M-M* chromosome, and were, therefore, *M-M+/M-M zl*. From the percent of *M-M* plants the percent of crossing over can be calculated. In F_2 from *M-M zl/W-R+*, the percent of *M-M* plants (homozygous dominants) in the whole population should vary from 0 to 25 as the percent of crossing over varies from 0 to 50. Similarly, in F_2 from *M-M zl/R-R+*, the percent of *M-M* plants (homozygous recessive) should vary from 0 to 25 with percent of crossing over varying from 0 to 50. From an observed percent of *M-M* plants in F_2, the percent of crossing over can be calculated by interpolation in a table prepared for the purpose. The available F_2 data for the two classes of heterozygotes, including records published earlier (EMERSON, BEADLE, FRASER 1935) are given in table 2.

TABLE 2

F_1 GENOTYPE	TYPES OF F_2 PLANTS				TOTAL	PERCENT *M-M*	PERCENT CROSSING OVER
	R-R	*M-M*	*M-R*	*W-R*			
M-M zl/R-R+	705	7	—	—	712	0.983	1.487
M-M zl/W-R+	—	28	1861	1012	2891	0.969	1.466
Total		35			3603	0.971	1.469

As shown in this table, the percent of crossing over between *P* and *zl* is 1.5. Since the crossover percent for *P ts* is 1.3 and for *P ms* 3.0, *zl* must be about 0.2 or 2.8 from *ts* and 1.5 or 4.5 from *ms*, depending on whether *zl* is on the same side of *P* as *ts* or *ms* or on the opposite side. The nature of the characters involved, zygotic lethal, tassel-seed, male-sterile, make it impossible to use the ordinary three-point back-cross test as a means of determining the exact loci of this group of genes, but fortunately another method can be employed.

LINEAR ORDER OF THE GENES: *P*, ms_{17}, AND *zl*

Since, to hand pollinate all plants involved in this study would require an inordinate amount of work and since crossovers are infrequent with genes so closely linked, it has usually happened that crossover plants had been open-pollinated before they were detected. But the close linkage renders crossover chromosomes relatively stable so that the new association of genes can be studied even though the plant first exhibiting it had been open pollinated. To avoid undue confusion, cultures produced by the selfed heterozygotes *M-M zl/W-R ms* and *M-M zl/W-R ts* were grown on the two sides of an isolated block. Since *ms* and *ts* plants produce little

or no viable pollen, the pollen produced in such isolated fields consists of a mixture of about 50 percent *M-M zl*, 25 percent *W-R ms*, and 25 percent *W-R ts*. This mixture is, of course, modified slightly by the pollen of the few crossover plants present in the block and by new crossover gametes of non-crossover plants. On one occasion, the open-pollinated block adjoined a field consisting largely of normal *W-R* and *W-W* plants.

In the populations making up these partly isolated blocks, three types of crossover plants can be detected by mere observation without resort to progeny tests. In cultures grown from selfed *M-M zl/W-R ms*, these three types are as follows:

I—Homozygous *M-M* plants, usually *M-M+/M-M zl*.
II—Male sterile *M-R* plants, usually *M-M ms/W-R ms*.
III—Normal *W-R* plants, usually *W-R+/W-R ms*.

These three crossover types will be considered one by one in an attempt to discover the linear order of the genes *P*, *ms*, and *zl*. Later similar types from cultures grown from selfed *M-M zl/W-R ts* will be considered.

There are only three possible linear orders of the genes *P*, *ms*, and *zl* as shown below for the heterozygote *M-M zl/W-R ms*:

Alpha	*Beta*	*Gamma*
+ *M-M zl*	*M-M zl* +	*M-M* + *zl*
ms W-R +	*W-R* + *ms*	*W-R ms* +

Type I crossovers, homozygous *M-M* plants, can arise only by replacement of *zl* by its dominant allele in at least one *M-M* chromosome. This might be accomplished by a crossover in region 2 of *alpha*, region 1 of *beta*, or in either region 1 or 2 of *gamma*. Since no one of these genes can be more than five units from any other one, it is fair to assume that double crossing over never or very rarely ever occurs. Without double crossing over, when *zl* is lost from the *M-M* chromosome, that chromosome could *never* acquire *ms* simultaneously if the order is that shown in *alpha*, must *always* acquire it if the order is that of *beta*, and might or might not acquire it if the order is that of *gamma*. The problem, therefore, is to determine whether on losing *zl* the *M-M* chromosome *never*, *always*, or *sometimes* picks up *ms*.

Type II crossovers, male-sterile mosaic-pericarp plants, might arise by a crossover in region 1 of *alpha* order, in either region 1 or 2 of *beta* order, or in region 1 of *gamma* order. Without double crossing over, when the *M-M* chromosome acquires *ms* it can *never* lose *zl*, if the order is that of *alpha*, may or may not lose it if the order is that of *beta* and must *always* lose it if the order is that of *gamma*. The problem is then to determine whether the *M-M* chromosome on acquiring *ms never*, *sometimes*, or *always* loses *zl*.

Type III crossovers, normal *W-R* plants, might arise from a crossover in region 1 of *alpha*, in either region 1 or 2 of *beta*, or in region 1 of *gamma*. Without double crossing over, when *ms* is lost from the *W-R* chromosome, that chromosome could *never* pick up *zl* if the order of genes is that of *alpha*, might or might not if the order is *beta*, and must *always* do so if the order is *gamma*. What is to be determined, therefore, is whether the *W-R* chromosome, on losing *ms*, acquires *zl never*, *sometimes*, or *always*.

These several alternatives are summarized for ready reference in the following tabular statement, table 3:

TABLE 3

CROSSOVER TYPE	CHROMOSOME EXCHANGE	GENE ORDER		
		ms P zl *alpha*	*P zl ms* *beta*	*P ms zl* *gamma*
I	*M-M* loses *zl* and acquires *ms*	never	always	sometimes
II	*M-M* acquires *ms* and loses *zl*	never	sometimes	always
III	*W-R* loses *ms* and acquires *zl*	never	sometimes	always

Evidence from crossover type I

In cultures coming from selfed heterozygotes *M-M zl/W-R ms*, 28 homozygous *M-M* plants were found and studied more or less adequately. All these plants had been grown in isolated or semi-isolated blocks from selfed seed and the seeds produced by them had been open-pollinated, the available pollen having been about 50 percent *M-M zl*, 25–50 percent *W-R ms*, and 0 to 25 percent *W-R ts*. Small F_1 cultures were grown from each of the 28 open-fertilized ears. In all there were 443 F_1 plants and all of them were normal, not a single one male-sterile. Since 25 to 50 percent of the pollen produced in the isolated block the preceding year was *ms*, it is highly probable that some *ms* plants would have appeared in F_1 if the *M-M* chromsome had gained *ms* simultaneously with the loss of *zl*.

A few plants of most F_1 cultures were selfed and F_2 progenies grown. Thirty selfed F_1's were homozygous *M-M*, presumably *M-M+/M-M zl*, and 16 were heterozygous *M-R*, presumably *M-M+/W-R ms*.

The F_2 progenies of the 30 *M-M* plants totaled 1139 individuals, of which 1100 were normal and 39 *ms*. For some of the 39 plants recorded as *ms* identification was uncertain because of injury to the tassels, but in most instances identification was probably correct. It is significant that all of the 39 *ms* plants occurred in six of the 30 F_2 cultures, the other 24 cultures having consisted of normal plants only. Moreover, 36 of the 39 *ms* plants occurred in four of the six cultures, the other two cultures having had only three *ms* plants. The distribution of normal and *ms* plants in the two classes of cultures is shown in table 4:

TABLE 4

NUMBER OF F_2 CULTURES	TYPES OF F_1 PLANTS		TOTAL	PERCENT MALE-STERILE
	NORMAL	MALE-STERILE		
Four	208	36	244	14.7
Two	91	3	94	3.2

The genotype of the F_1 plants which gave rise to the two F_2 cultures showing 3.2 percent of *ms* plants was probably *+M-M+/ms M-M zl*. The 3.2 percent of *ms* plants corresponds to a crossover percentage between *ms* and *zl* of 4.8,which is not far from the maximum of 4.5 calculated earlier from the linkage of *P ms* and *P zl*. But the 14.7 percent of *ms* plants in the other four cultures could hardly have come from an F_1 of that genotype, for 14.7 percent recessive corresponds to a crossover percentage of slightly more than 25. If the four F_1's were *+M-M+/ms M-M+*, 25 percent instead of about 15 percent of the F_2 population should have been *ms*. That the parents of all these F_1 plants were *+M-M+/+M-M zl* is suggested by the fact that sibs of four of these F_1's threw no *ms* plants in F_2. It seems probable that *ms* might have come from crossover pollen in the open-pollinated isolation block while the loss of *zl* had certainly occurred in the selfed plants one generation earlier.

The F_2 progenies of the 16 heterozygous *M-R* selfed F_1's grown from open-pollinated crossover *M-M* plants exhibited two strongly contrasting types of segregation, as shown in table 5:

TABLE 5

NUMBER OF F_2 CULTURES	TYPES OF F_2 PLANTS					TOTAL
	M-M+	*M-R+*	*M-R ms*	*W-R+*	*W-R ms*	
Nine	84	129	11	11	71	306
Seven	1	138	17	9	81	246

Records of the seven F_2 cultures are approximately those expected in F_2 from selfed F_1's of the genotype *+M-M zl/ms W-R+*. If the original crossover *M-M* plants were *M-M+/M-M zl* and if 25 to 50 percent of the pollen of the field was *W-R ms*, as postulated, about one-fourth to one-half of the F_1 *M-R* plants should have been *+M-M zl/ms W-R+*. That is, the crossover chromosome was not involved and these seven cultures are irrelevant to this discussion. The other nine F_2 cultures with 84 *M-M*, 140 *M-R*, and 82 *W-R* plants, the latter mostly *ms*, are about what should be expected from selfed F_1's of the constitution *+M-M+/ms W-R+*. The 11 *M-R ms* and 11 *W-R+* plants are to be regarded merely as the result of a relatively high crossover frequency in the F_1 plants. These nine cultures afford no evidence that the *M-M* chromosome acquired *ms* when it lost *zl*.

As a general statement, therefore, it is fair to conclude that, when the *M-M* chromosome loses *zl* by crossing over, it does not simultaneously pick up *ms* or that it very rarely does so. It follows from this that linear order *alpha, ms P zl*, is the most likely one but that order *gamma, M-M ms zl*, is perhaps a possibility. The most significant conclusion, however, is that order *beta, M-M zl ms*, is impossible because *ms* certainly is not *always* acquired with the loss of *zl*.

Evidence from crossover type II

In addition to homozygous *M-M* plants arising because of the loss of *zl* from an *M-M* chromosome, considered in the preceding discussion, there are found *M-R ms* plants (*M-M ms/W-R ms*) coming from the addition, by crossing over, of *ms* to the *M-M* chromosome. In the same open-pollinated blocks in which type I crossovers were found, 56 type II crossovers were identified. F_1 cultures from seed of all these crossover plants were grown and a few ears of each selfed or crossed with appropriate tester stocks. The F_1 cultures had a total of 754 plants, 445 of which were normal and 309 *ms*. This is in strong contrast with F_1 of type I crossovers in which no *ms* plants appeared.

The F_1 *ms* plants were mostly *M-R*, presumably *M-M ms/W-R ms*. The problem is to determine whether the *M-M ms* chromosome carries *zl* also. Several of these *ms* plants were pollinated by normal *M-R* plants of a tester stock, the genotype of which was known to be *+M-M zl/ms W-R+*. If the F_1 *ms* plants so crossed carried *zl* in the *M-M* chromosome, *zl M-M ms*, no *M-M* plants should appear in the progeny except from new crossovers by which *zl* might be lost. Two sharply separable types of progenies were obtained, 30 of one type and two of the other, as shown in table 6:

TABLE 6

NUMBER OF CULTURES	TYPES OF TEST-CROSS PLANTS						
	M-M+	*M-R+*	*M-M ms*	*M-R ms*	*W-R+*	*W-R ms*	TOTAL
Thirty	23	481	0	454	33	465	1456
Two	24	24	9	44	0	33	134

Of the 1456 plants of the 30 cultures, 958 had mosaic to 498 white pericarp, and 537 were normal to 919 male-sterile, not far from 2:1 and 1:2 ratios, respectively. This behavior, together with the occurrence of relatively few *M-M* plants, indicates clearly that the 30 F_1 plants from which these test-cross progenies came had both *zl* and *ms* in their *M-M* chromosomes. It follows, therefore, that the *M-M* chromosome did not, in these instances, lose *zl* when it acquired *ms*.

The other two cultures, however, gave strikingly different results. Of the 134 plants, 101 had mosaic and 33 white pericarp, a 3:1 ratio. And of the mosaic-pericarp plants, 33 were *M-M* and 68 *M-R*. Obviously the *M-M* chromosomes of these two F_1's carried *ms* but not *zl*. This situation might have occurred by the loss of *zl* simultaneously with the acquisition of *ms*, but this is not the only possibility. The acquisition of *ms* might have occurred in the selfed parents of some of the isolation-block plants and some of the gametes of the resulting plants in the isolation-block might have lost *zl*. In either case the *ms* parents of these two test-cross cultures must have been *ms M-M+/ms W-R+*.

So far only the *ms* plants appearing in the progenies of type II crossovers found in isolation blocks have been considered. If, in general, *zl* is retained by the *M-M* chromosome when it acquires *ms*, the normal plants grown from open-fertilized seed of type II crossovers should be of no significance in this discussion. If these plants are *ms M-M zl/ms W-R+* and are pollinated with a mixture of *+M-M zl* and *ms W-R+* pollen, the only normal plants of the next generation should be *ms W-R+/+M-M zl*. In short, the crossover plant contributes to these normal plants of the next generation only the non-crossover chromosome *ms W-R+*.

The F_1 progenies of open-pollinated type II crossovers contained normal *M-M* and *M-R* plants whose selfed progenies gave results of significance for the problem here under consideration. Of 26 F_1 normal *M-M* and *M-R* plants, in eight instances the crossover parent had obviously been pollinated in part with *W-R* ts_2 pollen, in seven with *W-R* pollen, and in 11 with *W-W* pollen. The resulting F_2 cultures of the three groups are given separately in table 7:

TABLE 7

NUMBER OF F_2 CULTURES	TYPES OF F_2 PLANTS								TOTAL
	M-M +	*M-M* *ms*	*M-R* +	*M-R* *ms*	*M-R* *ts*	*W-R* +	*W R* *ts*	*W-W* +	
Eight	0	1	138	34	2	18	80	—	273
Seven	0	1	226	14	—	123	—	—	364
Eleven	477	21	—	—	—	—	—	236	736

In the first group of eight and the second one of seven cultures from selfed *M-R* normals, only one *M-M* plant occurred in each; the respective numbers of plants with mosaic and with white pericarp were 175 to 98 and 241 to 123; and the respective numbers of normal and *ms* plants were 238 to 35 and 349 to 15. Evidently *zl* was here associated with *M-M* and *ms*, thus preventing, except by new crossovers, the appearance of homozygous *M-M* and *ms* plants and thereby resulting in 2:1 ratios of colored to white

pericarp. Such behavior is that expected from selfed plants of the respective genotypes *ms M-M zl/ts W-R+* and *ms M-M zl/+W-R+*. In the third group of 11 cultures from selfed *M-M* normals, there were 498 plants with colored to 238 with white pericarp, a 2:1 ratio; and there were 715 normal to 21 *ms* plants. Obviously the 11 selfed *M-M* plants had the genotype *ms M-M zl/+W-W+*.

In 56 of the 58 tests of type II crossovers, it is certain that the *M-M* chromosome did not lose *zl* when, by crossing over, it gained *ms*. In only two of the 58 tests, might *zl* have been lost at the same time that *ms* was acquired by this chromosome. And, even here, the loss of *zl* might have occurred one generation later than the acquisition of *ms*. These results indicate strongly that linear order *alpha, ms P zl*, is the correct one but do not disprove the possibility that the order might be that of *beta, P zl ms*. The most significant conclusion derived from these records is that order *gamma, P ms zl* is impossible, because certainly *zl* is not *always* lost when *ms* is gained by the *M-M* chromosome.

It will be recalled that the records from 37 F_2 cultures grown in the study of crossover type I, homozygous *M-M*, indicated order *alpha* as the most probable but did not exclude order *gamma*, while the records of the 58 cultures used in a study of crossover type II, *M-M ms*, indicated order *alpha* as most probable without excluding the possibility of order *beta*. But the crucial conclusions are that the type I crossovers studied could not possibly have come from order *beta* and that the type II crossovers studied would have been impossible with order *gamma*. The only possible order, therefore, is *alpha*, that is, with *P* located between *ms* and *zl*.

Evidence from crossover type III

Even though the evidence already presented from studies of crossover types I and II is conclusive, it is perhaps worth while to present the few data available from studies of type III crossovers, normal *W-R* plants. The problem here is to determine whether the *W-R* chromosome acquires *zl* on losing *ms* by crossing over. In the isolation blocks, *+M-M zl* and *ms W-R+*, pollen was abundant. If the *W-R* chromosome, on losing *ms* were to gain *zl*, gametes with the crossover chromosome *W-R zl* could produce no seed when fertilized with an *M-M zl* gamete. Yet, the progenies of open-pollinated *W-R* crossover plants consisted largely of normal *M-R* and *W-R* plants. Many of the *M-R* plants were, naturally, the result of fertilization of a non-crossover *W-R ms* egg by an *M-M zl* sperm and are of no importance in this study. Five selfed *M-R* plants, however, threw normal *M-R* and *W-R* plants in F_2, in all, 211 of the former and 108 of the latter. Certainly in these five instances *zl* was not acquired by the *W-R* chromosome when it lost *ms*. The 2:1 ratio and the absence of *M-M*

plants is, of course, brought about by the presence of *zl* associated with *M-M*. Six *W-R* plants in progenies of open-pollinated *W-R* normals were, evidently, the result of pollination with *W-W* pollen. Their F_2 progenies included 472 *W-R* and 145 *W-W* plants, all normals. This is approximately a 3:1 ratio, as is expected in F_2 from selfed plants of the constitution *+W-R+/+W-W+*. In these instances also the *W-R* chromosome did not gain *zl* on losing *ms*. This is the result expected with the *alpha* order of genes.

But one *M-R* and one *W-R* plant gave different F_2 results. The former produced 129 *M-R* to 16 *W-R* plants, as might have been expected if it was *W-R zl/M-M+*. The latter produced 117 *W-R* to 56 *W-W* plants, a 2:1 ratio, expected if it was *W-R zl/W-W+*. Such results can not be interpreted on the basis of a single crossover with the *alpha* order of genes, and *beta* and *gamma* orders have been shown to be impossible by studies of crossover types II and I, respectively. It is not necessary, however, to postulate double crossing over in this short region of chromosome 1. The F_1 plant, which gave in F_2 129 *M-R* to 16 *W-R* plants and which was presumably *W-R zl/M-M+*, had two *W-R* sibs in which the *W-R* chromosome did not carry *zl*. In this case and also in case of the F_1 *W-R* plant which gave 117 *W-R* to 56 *W-W* plants in F_2, the crossovers by which the *W-R* chromosome lost *ms* certainly occurred in the selfed parents of the isolation-block cultures in which the crossovers were first detected. If these two isolation-block plants had been *+W-R+/ms W-R+* the *+W-R+* chromosome could not have picked up *zl* by crossing over because its homolog, the commonly present *ms W-R+* chromosome, did not carry *zl*. If, however, these two plants were *+W-R+/ms W-R zl*, the first *W-R* chromosome of at least one gamete might have gained *zl* from the second *W-R* chromosome. But the genotype here postulated presupposes its formation by the union of two crossover gametes, *+W-R+* and *ms W-R zl*.

Moreover, the seed from which one of the F_1 plants grew must have been formed from fertilization with a crossover pollen grain, *+ M-M +*. Whether this concatenation of unusual events is less or more likely than double crossing over in a region of about five crossover units or than the occurrence of a new *zl* mutation, the problem does not affect the well grounded conclusion that the postulated *alpha* order of genes, *ms P zl*, is the actual one.

LINEAR ORDER OF THE GENES *P*, ts_2, AND *zl*

In an attempt to determine the linear order of the genes *P*, *ts*, and *zl*, the same method was used as in similar studies involving *ms*, already reported. Progenies of selfed plants of the genotype *+ M-M zl/ts W-R +* were grown at the side of a field made up largely of *+ W-R +* and *+ W-W*

+ plants, or in an isolated block containing on one side progenies of selfed plants of the constitution + *M-M zl/ms W-R* +. The plants were open-pollinated and, in the isolation-block, the available pollen consisted of approximately 50 percent *zl M-M* +, from 50 to 25 percent + *W-R ts*, and from 0 to 25 percent + *W-R ms*. Crossover types I and II and possible gene orders, *alpha*, *beta*, *gamma*, are the same as given in the preceding account involving *ms*, except that *ts* is substituted for *ms* in each case.

Evidence from crossover type I

Thirteen F_1 cultures were grown from open-pollinated plants of crossover type I, homozygous *M-M*. Of these F_1 plants, 286 were normal and none *ts*. This indicates that, when *zl* was lost from the *M-M* chromosome, *ts* was not gained. Thirty-five of these normals, 15 *M-M* and 20 *M-R*, were selfed and F_2 progenies grown from them. Records of like cultures are grouped together in table 8.

TABLE 8

NUMBER OF CULTURES	TYPES OF F_2 PLANTS									TOTAL
	M-M +	*M-M ts*	*M-R* +	*M-R ts*	*M R ms*	*W-R* +	*W-R ts*	*W-R ms*	*W-W* +	
Nine	5	—	154	5	—	7	94	—	—	265
Eight	56	1	88	—	—	6	49	—	—	200
Three	21	—	35	—	1	5	—	14	—	76
Ten	227	—	—	—	—	—	—	—	—	227
Five	115	—	—	—	—	—	—	—	28	143

The F_1 parents of the first group of nine cultures were, doubtless, *zl M-M* +/+ *W-R ts*. Evidently, the non-crossover chromosome, *zl M-M*, was involved here, and the records, therefore, are of no use in this discussion. The F_1 parents of the other four groups of eight, three, ten, and five cultures, apparently had, respectively, the genotypes + *M-M* +/*ts W-R* +, + *M-M* +/*ms W-R* +, + *M-M* +/+ *M-M zl* or + *M-M*+/+ *M-M* +, and + *M-M* +/+ *W-W* +. It is obvious that in none of the original crossovers concerned in these tests did the *M-M* chromosome, on losing *zl*, gain *ts*. This indicates the *alpha* order of genes or the *gamma* order with all the crossovers occurring in region 2. The most significant conclusion is that the *beta* order is impossible.

Evidence from crossover type II

Only nine open-pollinated crossover plants of type II, *M-R ts*, were studied. Their F_1 progenies consisted of 69 normal and 89 *ts* plants. Thirteen normal plants, one *M-M* and 12 *M-R* were selfed and seven *ts* plants, all *M-R*, were crossed by + *M-M zl/ts W-R* +. The records of the F_2 and test-cross progenies are presented in three groups in table 9:

TABLE 9

NUMBER OF CULTURES	TYPES OF F_2 AND TEST-CROSS PLANTS					TOTAL
	M-M +	*M-R* +	*W-R* +	*M-R ts*	*W-R ts*	
Twelve	1	130	4	4	70	209
One	32	—	—	—	—	32
Seven	2	77	2	71	76	221

The first group of 12 cultures, evidently, was derived from F_1 plants of the genotype + *M-M zl/ts W-R* +. The non-crossover *W-R ts*, not the crossover *M-M ts* chromosome, was involved. These records, therefore, are of no use in this study. The one F_2 culture might have come from an F_1 of the constitutions *ts M-M zl*/+ *M-M* +, and it could not have come from *ts M-M* +/+ *M-M zl*. The seven cultures from *M-R ts* F_1 plants crossed by + *M-M zl/ts W-R* + could have come only from *ts* plants of the genotype *ts M-M zl/ts W-R* +. All the evidence, therefore, indicates that *zl* is not lost when *ts* is acquired by a crossover in the *M-M* chromosome. This indicates that the *alpha* gene order is the correct one or that, if the *beta* order, all the crossovers occurred in region 2. The most significant conclusion is that the *gamma* order is impossible.

Since the evidence from crossover type I proves the *beta* order impossible and from type II shows the *gamma* order to be impossible, the actual order is the *alpha* one with *P* between *zl* and *ts*. This is the same order found for *ms*, *P*, and *zl*. The genes *ts* and *ms* must, therefore, be on the same side of *P*. The order for *ts* and *ms* can be inferred from the crossover percentages of 1.3 for *P ts* and 3.0 for *P ms*. Moreover, the one double recessive *ms ts* plant noted earlier could have arisen by a single crossover in the genotype from which it came only if the order of genes is *ms*, *ts*, *P*. The order of the four genes, therefore, is believed to be *ms ts P zl* or *zl P ts ms*.

POSSIBLE DOUBLE CROSSING OVER IN THE REGION *ms* TO *zl*

Here and there throughout the preceding account, crossover plants have been reported the genotypes of which might have arisen from a single crossover if the order of genes had been that of the postulated *beta* or *gamma* and could not have come from a single crossover with the *alpha* order which has been demonstrated to be the actual one. Any of these exceptional crossovers could be accounted for by double crossing over with the *alpha* order of genes, but, as has been shown, that assumption is not the only possible one. It has been pointed out that two separate crossovers, one in the open-pollination-block, where the crossover plants were discovered, and one in the preceding generation, furnished an adequate interpretation. In one instance, it was necessary even to assume the union of

two crossover gametes in one generation plus the functioning of a third crossover gamete in the next generation. Unlikely as this peculiar chain of events may be, it is perhaps quite as likely as the occurrence of a double crossover in a region of approximately 4.5 crossover units. One dislikes to postulate the occurrence of new mutations, though the possibility is not excluded.

One exceptional crossover plant, not mentioned previously, can not be dismissed so easily. The case, therefore, deserves somewhat detailed treatment. The exceptional behavior occurred in the tester stock involving the progeny of selfed + *M-M zl/ms W-R* +. A culture of this stock consisted of 55 *M-R* +, one *M-R ms*, and 26 *W-R ms* plants. Individual Number 10 of this culture, an *M-R* normal, was selfed and the resulting progeny contained 25 *M-R* +, two *M-M* +, and 14 *W-R ms* plants. All this is, of course, typical behavior for this stock. Pollen of this same Number 10 plant was used to pollinate a normal *R-R* plant and 40 normal *R-R* F_1 plants resulted. They were presumed to be + *M-M zl*/+ *R-R* +, and *ms W-R* +/+ *R-R* +. Four selfed *R-R* plants of this F_1 culture gave F_2 progenies such as expected on this assumption. Two of the four F_1 plants were, obviously, *ms W-R* +/+ *R-R* +, and need be given no further consideration. Two other F_1 plants were, doubtless, + *M-M zl*/+ *R-R* + as expected. Their F_2 progenies from selfing consisted of 181 *R-R* normals; and their F_1 progenies, when crossed on a normal *W-W* stock, had 93 *R-R* and 95 *M-M* plants.

One normal *R-R* of the original F_1 culture of 40, however, on being selfed produced an F_2 progeny of 184 *R-R* +, four *R-R ms*, one *M-M* +, and 58 *M-M ms*. The genotype of this F_1 must have been *ms M-M* +/+ *R-R* +. One gamete of individual Number 10 of the tester stock crossed on normal *R-R* must, therefore, have been *ms M-M* +. In short, when *zl* was lost (type I crossover) *ms* was gained, or, when *ms* was acquired (type II crossover) *zl* was not retained. There is no possibility that the genotype here concerned could have arisen by separate crossovers in plants of different generations, except the chance, unfortunately not always excluded, that a stray grain of pollen, instead of a pollen grain from individual Number 10 of the tester stock, functioned in the production of the one normal *R-R* plant which was responsible for this involved discussion. The almost insurmountable difficulty with the suggestion of the accidental functioning of a stray grain of pollen in the otherwise controlled cross-pollination is that, so far as known, no stock of maize in which *ms* was associated with *M-M* without *zl* was in existence at the time. In fact the first known occurrence of this association of genes was in the progeny of the selfed plant here under discussion. This leaves the alternatives of postulating (1) a mutation of normal to *ms* in the *M-M* chromosome coincidentally with the

TABLE 10

F_1 GENOTYPE	PARENTAL COMBINATIONS	RECOMBINATIONS			TOTAL
		REGION 1	REGION 2	REGIONS 1 AND 2	
P + *br* / + T 1-5b +	242	71 15.8%	108 24.1%	28 6.2%	449*
sr *P* + / + + T 1-5b	178	89 23.7%	88 23.5%	20 5.3%	375*
ms_{17} *P* + / + + T 1-5b	51	5 5.7%	28 32.2%	3 3.4%	87
ts_2 *P* + / + + T 1-5b	169	3 1.4%	39 13.7%	0	211
P + *br* / + T 1-5c +	195	60 18.1%	58 17.5%	19 5.7%	332*
sr *P* + / + + T 1-5c	80 50 —	21 17 —	39 14 —	6 1 —	146* 82 —
	130	38 16.7%	53 23.2%	7 3.1%	228
T 1-3a + + / + *br* bm_2	48	30 22.1%	37 27.2%	21 15.4%	136*
P + *br* / + T 1-3a +	104	20 10.3%	56 28.7%	15 7.7%	195*
sr *P* + / + + T 1-3a	43	21 27.6%	11 14.5%	1 1.3%	76*
ms_{17} *P* + / + + T 1-3a	87	2 2.0%	12 11.9%	0	101
ts_2 *P* + / + + T 1-3a	246	4 1.3%	65 20.6%	0	315
sr + *P* / + ms_{17} +	40	9 18.0%	1 2.0%	0	50
+ ms_{17} *P* / T 1-2c + +	309	60 15.7%	8 2.0%	4 1.0%	381
+ ts_2 + / T 1-2c + *P*	1175	249 17.4%	11 0.8%	1 0.1%	1436

* Unpublished data from E. G. ANDERSON.

crossover by which it lost *zl*, or a mutation of *zl* to its normal allele when *ms* was added by crossing over, (2) a somatic crossover in addition to a meiotic one, or (3) double crossing over in a region of less than five crossover units. If a double crossover did occur in this instance, it happened in a region shorter than that commonly given as the minimum for Drosophila.

ORIENTATION OF THE ms_{17} ts_2 *P zl* COMPLEX IN CHROMOSOME 1

It has been shown that the order of genes here under discussion is *ms ts P zl* or *zl P ts ms*. Orientation of this group with respect to other genes of the short arm of chromosome 1 of maize had to await the discovery of suitable markers near enough to *P* to be usable. Such markers are now available in certain reciprocal translocations involving the short arm of chromosome 1 (ANDERSON 1935). These translocations, T 1–2c, 1–3a, 1–5b, 1–5c, were furnished by DR. E. G. ANDERSON of California Institute of Technology, who has also given permission to use unpublished data included with other three-point back-cross records in table 10.

The data presented in the above table show that the breaks in T 1–5b, 1–5c, and 1–3a are to the right of *P*, between *P* and *br*. The data involving these three translocations show further that *sr*, ms_{17}, and ts_2 are to the left of *P*. Finally, they show that T 1–2c is to the left of ms_{17} and ts_2. This is in accord with back-cross data showing that T 1–2c is very near *sr*, as follows:

$$\frac{+ \quad \text{T 1–2c}}{sr \quad +} \quad + \text{T} \quad 151 \qquad ++ \quad 1 \qquad sr \text{ T} \quad 1 \qquad sr + \quad 144 \qquad = 297.$$

Crossover percent *sr*—T 1–2c = 0.7.

The available data place this group of genes of the short arm of chromosome 1 of maize in the following order:

$$sr \leftarrow ms_{17}\ 1.7\ ts_2\ 1.3\ P\ 1.5\ zl \rightarrow br.$$

The gene zb_4, reported by H. K. HAYES (unpublished) to be near *P*, has not been tested with ms_{17} or ts_2.

SUMMARY

A Bolivian maize with mosaic red pericarp and cob was found to have a recessive zygotic lethal, *zl*, closely linked with the pericarp-color gene, *P* and the near-by genes male-sterile 17, ms_{17}, and tassel-seed 2, ts_2. The effect of *zl* is to prevent homozygosis of genes with which it is closely linked, and thus to change a 3:1 to a 2:1 F_2 ratio when linked with a dominant gene or to prevent the occurrence of the one class when linked with a recessive gene.

The nature of the characters studied made impossible the use of ordinary methods of determining the linear order of the genes concerned. The method employed, based on the assumption of very rare or no double crossing over in so short a region, may be illustrated as follows: The three possible orders of the genes *P*, ms_{17}, and *zl* and the associations of the three genes in the heterozygotes studied are:

Alpha order	*Beta* order	*Gamma* order
$+ \quad P \quad zl$	$P \quad zl \quad +$	$P \quad + \quad zl$
$ms_{17} \quad + \quad +$	$+ \quad + \quad ms_{17}$	$+ \quad ms_{17} \quad +$

When, by crossing over, *P* loses *zl* it could never acquire ms_{17} if the gene order is *alpha* and must always acquire it if the order is *beta;* and when *P* gains ms_{17} it could never lose *zl* in the *alpha* order and must always lose it in the *gamma* order. Many tests of easily detectable crossover types demonstrated that *P* on losing *zl* does not gain ms_{17} and on gaining ms_{17} does not lose *zl*, thus eliminating the possibility of the *beta* and *gamma* orders. The postulated *alpha* order, ms_{17} *P zl*, is, therefore, the actual one. Similar tests involving ts_2 proved the order to be ts_2 *P zl*.

Tests with appropriate markers, mostly reciprocal translocations with chromosome 1 breaks near *P*, have shown that *zl* is to the right of *P* with ms_{17} and ts_2 to its left. The genetic map for this short region of the short arm of chromosome 1 is:

$$sr \leftarrow ms_{17}\ 1.7\ ts_2\ 1.3\ P\ 1.5\ zl \rightarrow br.$$

The occurrence of several crossover plants, which could not have resulted from a single crossover with the order of genes as given above, were readily accounted for by two separate crossovers in succeeding generations. The one exception to this was a plant the change in whose genotype could have occurred only (1) by a single crossover between two genes accompanied by mutation of a third gene, (2) by a single crossover preceded by at least one somatic crossover, or (3) by a double crossover in a genetic distance of approximately 4.5 crossover units.

LITERATURE CITED

ANDERSON, E. G., 1924 Pericarp studies in maize. II. The allelomorphism of a series of factors for pericarp colors. Genetics **9**: 442–453.

1935 Chromosomal interchanges in maize. Genetics **20**: 70–83.

EMERSON, R. A., BEADLE, G. W., and FRASER, A. C., 1935 A summary of linkage studies in maize. Cornell Univ. Agr. Exp. Sta. Memoir **180**: 1–83.

TRANSLOCATIONS IN MAIZE INVOLVING CHROMOSOME 8

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INTRODUCTION

THE linkage group associated with chromosome 8 of maize was the last one to be established. Only two genes, j_1 (japonica) and ms_8 (male sterile) are listed by EMERSON, BEADLE and FRASER (1935). One further gene, v_{16} (virescent) has since been added. MCCLINTOCK (1933) showed by means of a deficiency that j_1 was located in the distal portion of the long arm. This is in accord with BURNHAM'S studies on the translocation T8-9a (BURNHAM 1930, 1934).

LINKAGE DATA

BURNHAM (1934) has reported 38.4 percent crossing over between T8-9a and japonica, this being the average value for 414 backcross plants.

TABLE 1

Backcross progenies from combinations $\frac{T\ +\ +}{+\ ms_8\ j_1}$.

	NORMAL (Ms_8) OFFSPRING					PERCENT CROSSING OVER T-ms_8	MALE STERILE		TOTAL	PERCENT CROSSING OVER ms_8-j
	T++	+++	T+j	++j	TOTAL		$ms\ j$	ms +		
T3-8a	113	10	16	0	139	7.2	155	14	308	9.7
T3-8b	110	59	12	1	182	32.9	138	14	334	8.1
T4-8a	67	37	8	2	114	34.2	101	15	230	10.9
T6-8a	112	10	7	0	129	7.8	111	12	252	7.5
T8-9b	77	31	7	4	119	29.4	127	19	265	11.3
T8-10b	134	37	9	0	180	21.0	122	28	330	11.2
T8-10c	45	19	7	0	71	26.8	79	10	160	10.6

Data to be presented in this paper deal with translocations listed as T3-8a, b, T4-8a, T5-8, T6-8, T8-9b, T8-10a, b and c (ANDERSON 1935). Table 1 gives data on backcrosses involving both ms_8 and j_1. Since ms_8 plants do not produce pollen, all the backcrosses were made using pollen of the F_1. The ms_8 plants were not classified for semisterility. The T-ms_8 crossover values were computed from the normal (Ms_8) plants only; the

ms_8-j_1 values from the totals of all plants. All seven of these translocations show the same order T-ms_8-j, indicating that both genes must lie toward the end of the chromosome with j_1 nearest the end. In view of McCLINTOCK'S (1933) placement of j_1 in the distal part of the long arm, it may be stated that both genes lie well out in the long arm, with j_1 outermost. This will be amply verified when the correlation with the cytology is presented. Following the convention of orienting the linkage maps with the end corresponding to the short arm to the left, the map must read, from left to right, T4-8a—T3-8a—ms_8—j_1.

Additional data involving japonica but not male sterile are given in table 2. In general the percentages of recombination agree with the cor-

TABLE 2

Backcross progenies from combinations $\frac{T+}{+j_1}$.

	PARENTAL COMBINATIONS		RECOMBINATIONS		TOTAL	PERCENT RECOMBINATION
T3-8a	75	70	23	14	182	20.3
T3-8b	95	54	29	47	225	33.8
T4-8a	303	323	203	221	1050	40.4
T6-8a	288	249	37	58	632	15.0
T8-10a	230	261	93	75	659	25.5
T8-10c	140	154	39	36	369	20.3

responding percentages obtained from the normal (Ms_8) offspring in table 1. They may be compared as follows:

	Table 1	Table 2	Combined
T 3-8a	18.7	20.3	19.6
T 3-8b	39.0	33.8	36.1
4-8a	39.5	40.4	40.3
6-8a	13.2	15.0	14.7
8-10c	36.6	20.3	23.0

The only exception is T8-10c. The high value from table 1 is based on only 71 plants, the low value from table 2 on 369 plants. The recombination value for the total is 23.0 percent, which may be accepted as more nearly correct. The distance of T8-10c from ms_8 may then be estimated as about 14 or 15 units. T8-10a may also be estimated as about 15 or 16 units from ms_8. Likewise T8-9a may be estimated as about 30 units from ms_8 on the basis of data presented by BURNHAM (1934). It must be realized that these are estimates instead of measurements, and will be listed as such in correlating with the cytological observations.

CYTOLOGY

Observations at the prophase of meiosis have given the positions of the interchange in seven of the translocations studied. The positions are recorded in decimal fractions of the distance from the spindle attachment (ANDERSON 1938). Thus 8L.2 indicates chromosome 8, long arm, two-tenths of the distance from the spindle attachment to the end of the chromosome.

3-8a	3L.6	8L.8
3-8b	3L.1	8L.2
4-8a	4S.5 +	8L.1
6-8a	6L.5	8L.7
8-9b	8S.2	9L.8
8-10a	8L.6	10S.3 +
8-10c	8L.5	10S.4

Most of the chromosome-8 translocations were studied early and mostly from greenhouse material. The observations are somewhat less reliable than those of chromosome 9 (ANDERSON 1938), most of which were done more recently. In general the placements on chromosome 8 may be taken as reliable to about .2. The short arms of chromosomes 8 and 10 are very short so the placements 8S.2 and 10S.3 show simply that the interchange is in the proximal half of the short arm.

T5-8A

One translocation, T5-8a, has given such erratic distribution of progeny as to merit special attention. Pollen of two sister F_1 plants backcrossed on $ms_8\, j_1$ stock gave the distributions shown in table 3. The two cultures gave quite similar results showing a great deficiency of ms_8 plants. Where an

TABLE 3

Backcross progenies from plants of T5–8a of constitution $\frac{T\ +\ +}{+\ ms\ \ j}$ *and* $\frac{T\ ms\ \ j}{+\ +\ +}$.

PARENT	NORMAL (ms_8)				MALE STERILE			TOTAL OFF-SPRING	CROSSING OVER *ms-j*
	T++	+++	T+*j*	TOTAL	*ms j*	*ms* +	TOTAL		
11-171-18	88		8	96	11	2	13	109	9.2
11-171-20	161	1	18	180	50	7	57	237	10.5
combined	249	1	26	276	61	9	70	346	10.1
	+++	T++	++*j*		*ms*+	*ms j*			
15-921-4	146	13	12	171	108	7	115	286	6.6

equality of ms_8 to Ms_8 plants is expected, only about one fourth as many were obtained. From a later generation, a plant of constitution T ms_8 j/ + + + was used as pollen parent of a backcross (table 3). The results are similar but with less discrepancy between Ms_8 and ms_8 plants, and more crossovers between T and ms_8. Another plant of constitution Tms_8/ ++ on backcrossing gave 190 + +, 12 T +, and 106 male sterile plants. For all cultures the average crossover value for the T-ms_8 interval is 4.0 percent; for the ms_8-j interval 8.5 percent. The map order is clearly T-ms_8-j.

Three sister plants of a cross of T5-8a with japonica were backcrossed to japonica. The results are summarized in table 4. In the two cultures in

TABLE 4

Backcross progenies from three sister plants of T5–8a of constitution T/j.

PARENT	USED AS	PARENTAL COMBINATIONS		RECOMBINATIONS		TOTAL	PERCENT RECOMBINATION	T	+	PERCENT T
		T+	+j	Tj	++					
8534-3	♂	81	21	15	8	125	18.4	96	29	76.8
8534-5	♂	29	85	8	20	142	19.7	37	105	26.1
8534-12	♀	77	78	9	14	178	12.9	86	92	48.3

which pollen of the F_1 was used, unbalanced distributions were obtained. In the one culture the T plants were high in frequency, as in table 3. In the other culture the T plants were deficient. Otherwise the amount and type of unbalance resemble those in table 3. The one culture in which the F_1 was used as female parent gave a balanced distribution

Hybrids involving the chromosome-5 gene, *pr*, have sometimes given ratios of purple to red seeds diverging widely from expectations. These have been studied by BURNHAM (1936) and interpreted as due to one or more genes affecting differential fertilization or pollen tube growth. Since T5-8a likewise involves chromosome 5, the unbalanced distributions ob-

TABLE 5

Reciprocal outcrosses of T5–8a/pr bm₁ plants to recessive pr.

PLANT NUMBER	AS PISTILLATE PARENT		AS POLLEN PARENT	
	Pr	*pr*	*Pr*	*pr*
18-289-2	84	90	24	92
18-289-6	126	112	24	151
18-289-8	117	90	92	174
			87	116
Total	327	292	227	533

tained are probably due to the same cause. The distributions of purple and red seeds in reciprocal outcrosses of three T5-8a/*pr* bm_1 plants (table 5) are in agreement with such an interpretation. In each case an approximate 1:1 ratio was obtained when used as female parent, while as pollen parent there was a deficiency of purple seeds.

CORRELATION OF CYTOLOGICAL POSITION WITH CROSSING OVER FROM ms_8

Table 6 presents a list of the translocations arranged in approximate order of their observed cytological positions together with their observed or

TABLE 6

Comparison of position of interchange with percent of recombination with ms_8.

	CYTOLOGICAL POSITION	RECOMBINATION WITH ms_8	NUMBER OF PLANTS IN LINKAGE TESTS	
			ms_8	j_1
T8–9b	S.2	29.4	119	119
T4–8a	L.1	34.2	114	1164
3–8b	L.2	32.9	182	407
8–9a	L.2	(30.0)*		(BURNHAM 1934)
8–10b		21.0	180	180
8–10c	L.5	(14.0)*	71	440
8–10a	L.6	(15.0)*		659
6–8a	L.7	7.8	129	71
3–8a	L.8	7.2	139	321
5–8a		4.0	649	791

* Estimated from T-j_1 recombinations.

estimated percentage of recombination with ms_8. The correlation is remarkably close considering the small number of plants and the difficulties involved in the cytological observations. The reliability of the cytological placements is not greater than .2.

The two genes ms_8 and j_1 appear to be in the terminal one-fourth of the long arm of chromosome 8, with j_1 nearest the end. Except perhaps for the gene v_{16}, the short arm and the proximal three-fourths of the long arm are devoid of known genes.

SUMMARY

Data are presented on linkage relations with ms_8 and j_1 for nine translocations involving chromosome 8. The cytological positions of seven of these have been determined.

The genes ms_8 and j_1 are in the terminal one-fourth of the long arm of chromosome 8, with j_1 nearest the end.

LITERATURE CITED

ANDERSON, E. G., 1935 Chromosomal interchanges in maize. Genetics **20:** 70–83.
1938 Translocations in maize involving chromosome 9. Genetics **23:** 307–313.

BURNHAM, C. R., 1930 Genetical and cytological studies of semisterility and related phenomena in maize. Proc. Nat. Acad. Sci. **16:** 269–277.

BURNHAM, C. R., 1934 Cytogenetic studies of an interchange between chromosomes 8 and 9 in maize. Genetics **19:** 430–447.

BURNHAM, C. R., 1936 Differential fertilization in the *Bt Pr* linkage group of maize. Jour. Amer. Soc. Agronomy **28:** 968–975.

EMERSON, R. A., BEADLE, G. W., and FRASER, A. C., 1935 A summary of linkage studies in maize. Cornell Univ. Agr. Exp. Sta. Memoir **180:** 1–83.

MCCLINTOCK, BARBARA, 1933 The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. Z. Zellf. mik. Anat. **19:** 191–237.

GENETICS OF NATURAL POPULATIONS. IV. MEXICAN AND GUATEMALAN POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

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INTRODUCTION

NOTWITHSTANDING their external uniformity, free-living populations of *Drosophila pseudoobscura* are replete with genetic variability. The variations in the shape of the Y chromosome (DOBZHANSKY 1935, 1937), and in the gene arrangement in the third chromosome (DOBZHANSKY and STURTEVANT 1938) are especially striking. A genetic analysis of the third chromosomes has shown that a considerable proportion of them carry recessive lethals, semilethals, deleterious viability modifiers, and other morphological and physiological changes (STURTEVANT 1937, DOBZHANSKY and QUEAL 1938b).

The present study was undertaken in order to extend the above observations by using samples of the populations of *D. pseudoobscura* inhabiting the highlands of Mexico and Guatemala. A greater comprehensiveness was, however, not the only aim of the work. In the absence of suitable techniques of creating experimental populations of Drosophila, the general problems of population mechanics can be attacked only through comparative analysis of judiciously selected free-living populations. For reasons to be explained below, a comparison of Mexican and Guatemalan populations with those from the temperate part of North America seemed especially promising.

MATERIAL AND TECHNIQUE

The distribution area of *D. pseudoobscura* extends from the boreal forests of British Columbia to well below the Tropic of Cancer. In Mexico and Guatemala it inhabits the montane coniferous and broad leaved forests, within the altitudinal limits of approximately 5500–8500 feet. It does not occur in the tropical lowlands, even when pine groves are encountered therein (for example, south of Chilpancingo, Guerrero, Mexico, or near Quirigua, Guatemala). Its south-eastern limit is apparently reached in the highlands of western Guatemala; at least, it has not been found in the pine barrens around Guatemala City which appear, judging by analogies, not unfavorable for it. A list of the localities in which population samples were collected is given below (compare with figure 3). The names used to designate the strains are italicized.

Guatemala: Finca Santa Victoria, near Panajachel, Lake *Atitlan*, Feb-

ruary 9, 1938, mixed wood; *Totonicapan*, February 11, pine grove; slope of Santa Maria, at Zunil, near *Quezaltenango*, February 12, pine wood; between San Francisco and *Momostenango*, February 13, pine wood; Salegua valley, near *Huehuetenango*, February 14, mixed wood; Rancho Tejor, near *Sacapulas*, February 15, mixed forest; *Chichicastenango*, February 16, deciduous grove.

Mexico, *Oaxaca:* Cerro San Jose, near Ejutla, September 1936, mixed forest; Morelos: north of *Cuernavaca*, September 1936 and March 7, 1938, mixed forest. Mexico: foot of Iztaccihuatl, near *Amecameca*, March 9, pine wood. Michoacan: Lengua de la Vaca, near *Zitacuaro*, March 13, pine wood; Quiroga, Lake *Patzcuaro*, March 14, mixed wood; east of *Morelia*, March 15, oak grove. Vera Cruz: mountains above Tecamaluco, near *Orizaba*, March 20, mixed wood. Puebla: below Asseraderos de San Felipe, east of *Tehuacan*, March 22, pine grove; slope of Iztaccihuatl, northwest of *Puebla*, March 23, mixed forest. Hidalgo: Omitlan, near *Pachuca*, March 25, mixed grove. Durango: Rancho *Otinapa*, September 1936, pine forest.

The wild females trapped were isolated from the males, shipped to the Laboratory at Pasadena, and each of them became the progenitor of a separate strain. Chromosomes of the larval salivary glands were examined in temporary aceto-carmine mounts; the larvae to be used for this purpose were grown at 18°–20°C, with yeast added to the regular medium. The shape of the Y chromosome was studied in temporary aceto-carmine smears of the testicular tissue of freshly hatched adult males. The crosses made for the analysis of the gene contents in the third chromosomes were raised generally at room temperature, but the generations (F_3, F_4, and F_5) in which the counts were made were always kept in an incubator at 24.5°C.

GENE ARRANGEMENTS IN THE THIRD CHROMOSOME

Seventeen distinct gene arrangements, related to each other mostly as overlapping inversions, were identified in the third chromosome of *D. pseudoobscura* by DOBZHANSKY and STURTEVANT (1938). With the aid of a method described in detail in the paper just referred to, and which need not be dwelt upon here, the phylogenetic relationships of these seventeen structural types were established (compare with the phylogenetic chart in figure 3 of that paper). The arrangements denoted as Standard, Hypothetical (resembling that found in *D. miranda*) and perhaps Santa Cruz, are the ancestral ones. Standard has given rise to a family of related types which occur mostly in race B, but two of which, namely Arrowhead and Pike's Peak, are restricted to race A. Race B of *D. pseudoobscura* does not occur in Mexico and Guatemala. Santa Cruz has produced a family of four arrangements: Cuernavaca, Chiricahua I, Mammoth, and Tree Line. Tree Line has, in turn, given rise to a family of four derivatives: Estes Park,

Oaxaca, Olympic, and Hidalgo. Santa Cruz, Tree Line, and their derivatives are found exclusively in race A.

The identification of the gene arrangements is technically easiest if the individual to be tested is outcrossed to another whose third chromosomes are known beforehand. The chromosomes are examined in the salivary glands of the larvae in the F_1 of the cross. If the individual in question has the same gene arrangement in its chromosomes as that present in the tester strain, only simple chromosome strands are found in the hybrid. Any difference in the gene arrangement is reflected in formation of aberrant pairing configurations. If, finally, the tested individual is a structural heterozygote, two or more types of hybrid larvae are encountered. To

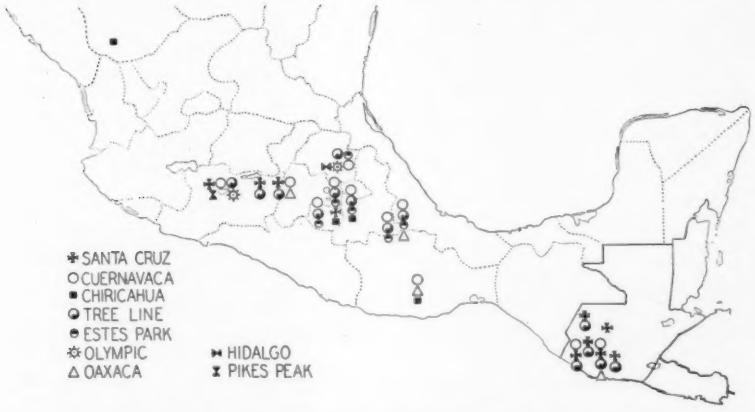

FIGURE 1.—The distribution of the structural types of the third chromosome in Mexico and Guatemala.

facilitate the observation, tester strains are so chosen that the gene arrangement found in them resembles as much as possible that expected in the materials tested.

Two strains, one homozygous for the Santa Cruz and the other for the Tree Line arrangements in the third chromosomes, were used as testers for Mexican and Guatemalan populations. Single sons of the females caught outdoors, and in some instances single grandsons of such females, were crossed to females from the tester strains. A summary of the data thus obtained is presented in table 1 and in figure 1.

Nine gene arrangements in the third chromosome are recorded in the material examined. In three localities from which adequate samples were available (Pachuca, Amecameca, and Patzcuaro), as many as five structural types were detected in the same population. This indicates that

Mexican and Guatemalan populations of *D. pseudoobscura* tend, on the whole, to be more heterogeneous with respect to the gene arrangement than is the rule in populations from more northern latitudes (DOBZHANSKY and STURTEVANT 1938). The total number of the arrangements recorded in race A from its entire distribution area, extending from British Columbia to Guatemala, is only thirteen. Among the nine found in Mexico, eight

TABLE 1

Frequencies of the gene arrangements in the third chromosome in populations from various localities in Mexico and Guatemala.

	SANTA CRUZ	CUERNAVACA	CHIRICAHUA	TREE LINE	ESTES PARK	OLYMPIC	OAXACA	HIDALGO	PIKE'S PEAK	CHROMOSOMES TESTED
Orizaba	—	27	—	5	4	—	4	—	—	40
Tehuacan	—	11	—	2	3	—	—	—	—	16
Pachuca	—	45	—	27	2	14	—	2	—	90
Puebla	—	13	1	11	5	—	—	—	—	30
Amecameca	1	17	2	11	19	—	—	—	—	50
Cuernavaca	—	4	—	—	—	—	—	—	—	4
Zitacuaro	2	9	—	4	—	—	1	—	—	16
Morelia	2	—	—	4	—	—	—	—	—	6
Patzcuaro	18	8	—	2	—	2	—	—	2	32
Quezaltenango	35	6	—	15	—	—	—	—	—	56
Momostenango	1	—	—	1	—	—	—	—	—	2
Totonicapan	9	1	—	3	—	—	1	—	—	14
Huehuetenango	5	—	—	1	—	—	—	—	—	6
Sacapulas	4	—	—	—	—	—	—	—	—	4
Atitlan	2	1	—	1	—	—	—	—	—	4

were known previously, and one, Hidalgo, is new. Hidalgo/Tree Line heterozygotes have a single short inversion in the third chromosome, which includes sections 77B, 78, 79A, 68D, 74AB, 73, 72C, and a part of 72B (figure 2). The position of Hidalgo in the phylogenetic scheme is, accordingly, among the Tree Line derivatives.

The presumed ancestral arrangement, the Standard, is common on the Pacific Coast of the United States, from British Columbia to Lower California, and occurs sporadically further east, as far as the Rockies and possibly Texas. Yet, Standard does not penetrate Mexico and Guatemala. The same negative conclusion must be reached with respect to Arrowhead, which is the commonest type almost everywhere in the United States where the species is found. The sole derivative of Standard that is found in Mexico, and at that very rarely, is Pike's Peak, which is rather common in the Rockies and very common in Texas. Pike's Peak is not found on the

Pacific Coast of the United States, except in a small area north of San Francisco Bay, where it has been detected in two samples collected by DR. A. H. STURTEVANT (from Sebastopol and from Hopland, California). All other gene arrangements inhabiting Mexico and Guatemala belong to the Santa Cruz and the Tree Line "families"; furthermore, all the members of these "families" are represented in these countries, with the single exception of Mammoth which is endemic to the Sierra Nevada of California. On the other hand, only three of the arrangements found in Mexico and Guatemala, namely Cuernavaca, Oaxaca, and Hidalgo, are endemic, while the remainder are found also in the United States, especially in California and in the Rocky Mountains of northern Colorado, although none of them

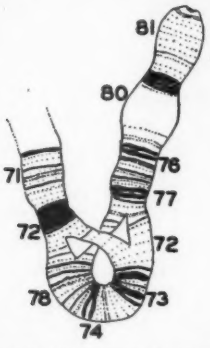

FIGURE 2.—The distal part of the third chromosome in the Tree Line / Hidalgo heterozygotes.

are known to go as far north as British Columbia. To summarize: populations inhabiting the extremes of the species area, British Columbia in the North and Mexico and Guatemala in the South, have no structural types in the third chromosome in common; populations from the intervening territories contain mixtures of the northern and the southern types in varying proportions.

Within Mexico and Guatemala the populations are also not uniform. The localities studied fall into two large groups. The first embraces east-central Mexico, from Orizaba to Cuernavaca (table 1), and the second includes west-central Mexico and Guatemala. The first group is characterized by high frequencies of Estes Park and Cuernavaca arrangements, and by the absence of Santa Cruz. A high frequency of Santa Cruz, a lower one of Cuernavaca, and absence of Estes Park are characteristic for the second group. The writer is insufficiently familiar with zoo- and phytogeography and geology to visualize the causal background of the division just described.

The geographical regularities discussed above represent what one could

perhaps call gross distributional features. Superimposed on them are much more local, microgeographic, variations, which manifest themselves primarily in unexpectedly large differences between populations inhabiting neighboring localities. Thus, 15.5 percent of the chromosomes at Pachuca have the Olympic arrangement, which is not found at all in other localities studied in east-central Mexico, and which reappears only in the relatively remote Patzcuaro. Although many samples are admittedly small, the statistical probability of the difference between Pachuca and adjacent localities being due to chance is negligible. A similar, though less pronounced difference exists between the Puebla and Amecameca samples, which come from places separated only by the northeastern spur of the volcano Iztaccihuatl, the crest of Rio Frio, which is completely covered with forest favorable for the habitation of *D. pseudoobscura*. Nevertheless, the Estes Park arrangement is more common at Amecameca than it is at Puebla; the χ^2 of the difference equals 4.062, which means that such or a greater difference may occur by chance less than once in twenty trials. The failure to find the Oaxaca arrangement anywhere in central Mexico except in Orizaba and Zitacuaro is also suggestive, although this may well be due to inadequacy of the samples. In general, although any one of such differences could conceivably be due to sampling errors, the combined data prove the existence of microgeographic variations rather convincingly. Whether such variations are permanant, in the sense that a population retains its characteristics from year to year, or are fleeting waves in the genetic composition of a local colony, can not be decided for the Mexican samples. DOBZHANSKY and QUEAL (1938a) and KOLLER (1939) found that populations inhabiting different mountain ranges in the Death Valley region of California, and even parts of the same range, are not alike. According to KOLLER, the composition of the population in a locality may differ in successive years.[1]

Somewhere between the macro- and the microgeographic variations stand the phenomena of discontinuity in the distribution of certain arrangements. The Chiricahua arrangement is rather common in the southwestern United States; the single strain known from northern Mexico (Durango) proved to be homozygous for it. Yet, in central Mexico this ar-

[1] The finding of DOBZHANSKY and QUEAL that populations inhabiting adjacent mountain ranges differ in composition, and that there are no clear geographical trends or "gradients" in these variations, has been misunderstood by some colleagues as implying a denial of the usual rule that such trends are present in intraspecific geographical variability. This is, of course, a misapprehension. The geographical trends certainly exist, and can be detected if samples from sufficiently remote localities are studied (compare with DOBZHANSKY and STURTEVANT 1938). But if samples are taken from close localities, the major trends are obscured by the microgeographic variations. The macro- and the microgeographic variations are not mutually exclusive, but, on the contrary, complementary, and the latter may prove to be the germs of the former.

rangement is rare (table 1). The two strains known from southern Mexico (Oaxaca) both contained it, and in Guatemala it has not been found. Estes Park is common in east-central Mexico, but not in the west-central part or in Guatemala. The only other area in which it is known to exist is the Rocky Mountains of northern Colorado. Olympic is known from the Olympic peninsula, Washington, from Mount Whitney, California, and from Pachuca and Patzcuaro, Mexico. The absence of Estes Park in west-central Mexico and in Guatemala appears to be a somewhat special case. A glance at table 1 or figure 1 shows that there is a kind of repulsion between the Estes Park and the Santa Cruz arrangements: they practically never occur together. Phylogenetically, Estes Park is descended from Tree Line, which is in turn a derivative of Santa Cruz. A comparison of Estes Park and Santa Cruz (compare with DOBZHANSKY and STURTEVANT 1938) reveals that these gene arrangements are very similar: the inversion transforming Tree Line into Estes Park has nearly undone the change produced by the inversion through which Tree Line arose from Santa Cruz. The Estes Park/Santa Cruz heterozygotes have in their salivary gland cells almost completely paired third chromosomes, showing only small deficiency-duplication buckles in the short sections where the homology is incomplete. STURTEVANT (1938) has shown that, on theoretical grounds, such inversions are not expected to coexist in the same population, since the crossing-over in the heterozygotes will give rise to gametes with unbalanced gene complements. According to STURTEVANT, this situation may serve as a starting point for the development of isolating mechanisms, and hence for species divergence. The "repulsion" between Estes Park and Santa Cruz observed in Mexican and Guatemalan populations may be regarded as evidence corroborating STURTEVANT's theoretical deductions.

VARIATION IN THE Y CHROMOSOME

Seven distinct types of Y chromosome are known in *D. pseudoobscura* (DOBZHANSKY 1935, 1937), four of which, types IV, V, VI, and VII, occur exclusively in race A, two, types II and III, only in race B, and one, type I, in both races. The shape of the Y chromosome has been examined in 26 strains from Mexico and in 9 strains from Guatemala. The results obtained are as follows.

Type I. Mexico. Durango: Otinapa-7. Michoacan: Patzcuaro-1, Patzcuaro-2, Zitacuaro-3. Morelos: Cuernavaca-5. Puebla: Tehuacan-1. Oaxaca: Cerro San Jose-4, Cerro San Jose-5.

Type IV. Mexico. Durango: Otinapa-3. Michoacan: Patzcuaro-4, Morelia-1, Morelia-3, Zitacuaro-2. Hidalgo: Pachuca-2, Pachuca-5. Mexico: Amecameca-1, Amecameca-3. Morelos: Cuernavaca-2, -5, -6, -8, -11. Puebla: Puebla-1, Puebla-2, Tehuacan-2. Vera Cruz: Orizaba-2, Orizaba-3.

Type VII. Guatemala: Quezaltenango-1, 2-, -3, Momostenango-1, Huehuetenango-1, Sacapulas-1, Chichicastenango-1, Totonicapan-1, Atitlan-1.

A summary of the above data is presented in figure 3 in map form. Types I and IV occur throughout Mexico, the former apparently being commoner in the western and the latter in the eastern part of the country. Beyond the confines of Mexico, type I has been recorded in race A from a single locality only, namely from Santa Rita Mts., which is close to the Mexican border. Type IV is found along the Pacific Coast of the United States and Canada, and also in Arizona and New Mexico. Type V, which is the commonest in the Rocky Mountain area, does not occur in Mexico at all. This negative information is interesting since it parallels the results obtained with the gene arrangements related to Standard in the third chromosome (see above).

FIGURE 3.—The distribution of the types of Y chromosome in Mexico and Guatemala.

A totally unexpected finding is that the Guatemalan populations are uniform in having the type VII of the Y chromosome. This type has been recorded previously only from two localities in northern Colorado, and from nowhere else in the United States or in Mexico. The possibility that we are dealing with two distinct but mimic types, one endemic to Guatemala and the other to Colorado, should not be disregarded: small differences in the metaphase chromosomes are easily overlooked. But the great difference between the Mexican and the Guatemalan populations with respect to the Y chromosome is also unexpected, since no analogous difference exists with respect to the gene arrangements in the third chromosome. The strains from Cerro San Jose, Oaxaca, which were recorded in 1937 as having the type I of the Y were reinvestigated, in order to ascertain that these strains have actually type I, and not VII, of the Y. The original

identification proved to be correct. The boundary between the Mexican and the Guatemalan populations is probably the low Isthmus of Tehuantepec, which, being covered with tropical vegetation, is in all likelihood not inhabited by *D. pseudoobscura*.

GENE ARRANGEMENT IN CHROMOSOMES OTHER THAN THE THIRD

The "sex-ratio" condition in race A of *D. pseudoobscura* (STURTEVANT and DOBZHANSKY 1937) is now known to be associated with a triple inversion in the right limb of the X chromosome. These inversions were repeatedly observed in preparations of the Mexican material, but estimates of the frequency of "sex-ratio" in populations can be made more easily by observing the progeny from the crosses of single sons of wild mothers to unrelated females. "Sex-ratio" males produce progenies consisting of daughters, and few or no sons. Sometimes two sons of the same female were tested; since a female has two X chromosomes, her sons may be either alike or different. If a single son is tested, the presence or absence of the "sex-ratio" in a single X is determined; if a second son of the same female is tested, the probability that the second X of that female is recovered is 50 percent. Therefore, it was considered that 1½ chromosomes were tested wherever the progeny of two brothers was observed. In east-central Mexico (the first six localities in table 1) 24½ "sex-ratios" were found among 126½ tested chromosomes, so that the frequency is 19.4 percent. In west-central Mexico (Zitacuaro, Morelia, and Patzcuaro) the figures are 5½ among 32, or 17.2 percent, and in Guatemala 1 in 65½, or 1.6 percent. The figure for Guatemala is astonishingly low.

In the chromosomes other than the third and the right limb of the X no inversions or other changes were detected, although in the older material from Cuernavaca a fourth chromosome inversion has been seen (DOBZHANSKY and STURTEVANT 1938).

GENIC VARIABILITY IN THE THIRD CHROMOSOME

Analysis of the population samples from the mountains of the Death Valley region has shown that about 15 percent of the third chromosomes carry recessive lethal or semilethal genes. Among the remaining 85 percent, a very large fraction carry viability modifiers, a decided majority of them unfavorable to the organism. Other types of hereditary variability, such as mutations producing visible external effects and modifiers of the rate of development, are also encountered (DOBZHANSKY and QUEAL 1938b). To obtain comparable data for Mexican and Guatemalan populations, the technique used previously in the work with those from Death Valley was followed.

Each female caught outdoors was allowed to produce offspring. A single

male was taken from every culture, and crossed to females homozygous for the third-chromosome recessives orange and purple (*or pr*). In the F_1, males were taken, one per culture, and crossed to females having *or*, Blade, Scute, and *pr* in one chromosome and the Cuernavaca inversion in the other. The inversion is used to prevent crossing over in the *or Bl Sc pr / Cuernavaca* females. In the next generation, denoted F_2 although it is not that strictly speaking, females and males showing *Bl* and *Sc* are selected from each culture and intercrossed; their constitution is evidently *or Bl Sc pr / wild*. In the F_3 a segregation in the ratio 66.7 percent *Bl Sc* : 33.3 percent wild type is expected, provided the wild chromosome in question carries no viability modifiers. A more detailed description of this technique, including a discussion of its limitations, may be found in the paper by DOBZHANSKY and QUEAL (1938b). Suffice it to add here that, since the *or Bl Sc pr* chromosome has the Standard gene arrangement and the wild chromosomes from Mexico and Guatemala always have arrangements other than the Standard, few or no crossovers appear in the offspring of *or Bl Sc pr* females.

Altogether 120 wild chromosomes, 38 from Guatemala and 82 from Mexico were analyzed. The proportion of the wild-type individuals in different F_3 cultures varied from zero to a high value of 57.05 percent (table 2). Complete absence of wild-types in a culture indicates that the wild third chromosome involved carries a lethal gene, or genes. The number of such cultures in the Guatemalan samples amounts to 18.4 percent, in the Mexican, to 22.0 percent and in the combined data to 20.83 ± 2.50 percent of the total. This may be compared with the figure for the Death Valley populations, which is 11.90 ± 0.75 percent. The infestation with lethals is greater in the Mexican and Guatemalan populations, the difference being significant.

In some cultures a few wild-types do appear, but their frequency is far below the theoretically expected 33.3 percent. Such cultures contain semilethals. The distinction between the lethals and the semilethals, and between the semilethals and the "normal" viability range, is not sharp. Arbitrarily we may consider any culture producing less than one-half of the expected number, that is, less than 16 percent, of wild-types as having a semilethal. Although this limit is an arbitrary one, shifting it a little upward or downward fails to alter the results appreciably, since cultures giving frequencies of wild-types in the neighborhood of 16 percent are not numerous. On this basis, the proportion of the chromosomes carrying lethals or semilethals in Guatemalan populations turns out to be 34.21 ± 5.20 percent, in the Mexican 28.05 ± 3.35 percent, and in the combined data 30.00 ± 2.82 percent. The comparable figure for the Death Valley populations is 14.96 ± 0.82; the difference is 15.04 ± 2.94 per cent, or five

times greater than the probable error. STURTEVANT (1937) recorded 19.25 ± 1.95 percent of lethals, and no semilethals, in samples from various parts of the distribution area of the species, including some Mexican chromosomes. Comparing this figure with ours for lethals and semilethals combined, which is entirely fair since STURTEVANT'S failure to discover semilethals is due clearly to chance, we find that Mexican and Guatemalan populations have 10.75 ± 3.43 percent more lethal-bearing chromosomes than the species at large, and hence the germ plasm of the Mexican and Guatemalan populations is infested with these genetic variants to a greater extent than is the case in other territories. With 30 percent of the third

TABLE 2

Frequency of wild-types (in percent) in the F_3 cultures; further explanation in text.

LOCALITY	0	0+1-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	20-22	22-24	24-26	26-28	28-30	30-32	32-34	34-36	36-38	...	57.05	TOTAL
Quezaltenango	3	3	—	—	—	—	1	—	1	—	—	—	1	—	4	3	4	2	1	1	...	—	24
Totonicapan	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	1	2	1	1	—		—	6
Momostenango	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	1
Sacapulas	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—		—	2
Huehuetenango	1	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—		—	3
Chichicastenango	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	1
Atitlan	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	1
Total Guatemala	7	3	—	—	1	—	1	—	1	—	—	—	1	1	5	5	7	3	2	1		—	38
Patzcuaro	1	1	—	—	1	—	—	—	—	—	—	—	—	—	3	—	4	1	1	—		—	12
Morelia	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—	1
Zitacuaro	2	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	—	—	—	—		—	4
Cuernavaca	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1		—	2
Amecameca	4	—	—	—	—	—	1	—	—	—	—	—	1	2	2	4	2	1	2	3		—	22
Puebla	3	—	—	—	—	—	—	—	—	1	—	—	—	—	—	2	—	2	—	2		1	11
Tehuacan	1	—	—	—	—	—	—	—	1	—	—	—	—	—	1	1	—	—	1	—		—	5
Orizaba	3	—	—	—	—	—	—	—	—	—	—	—	2	—	1	1	—	—	1	1		—	9
Pachuca	2	1	—	—	—	—	—	—	—	1	1	—	1	3	1	—	—	3	3	—		—	16
Total Mexico	18	2	—	—	1	—	1	—	1	2	1	—	5	5	8	9	6	7	8	7		1	82
Grand Total	25	5	—	—	2	—	2	—	2	2	1	—	6	6	13	14	13	10	10	8		1	120

chromosomes carrying lethals and semilethals, only half of the flies are free of them, 42 percent have one, and 9 percent have two lethal-bearing third chromosomes (this, of course, does not take into account the infestation which is probably present in the second, fourth, fifth, and X chromosomes).

Disregarding the lethals and the semilethals, a residue is obtained in which the frequency of wild-types varies from 16 percent to 38 percent, with one culture giving as much as 57 percent (table 2). To test whether this variation is genetic or is due to a combination of environmental influences with sampling errors, a F_4 generation was raised. For this purpose, *Bl Sc* females and males were selected in F_3 cultures and inbred; counts in the

offspring were made to determine the frequencies of wild-type and *Bl Sc* individuals. The results are shown in figure 4 in form of a correlation table, comparing the frequencies of the wild types in the F_3 and F_4 cultures of the same strains. The strain that gave 57.05 percent of wild types in F_3 is omitted; three F_4 cultures were raised from this line, giving respectively 29.3 percent, 33.1 percent, and 34.9 percent wild-type. The unreasonably

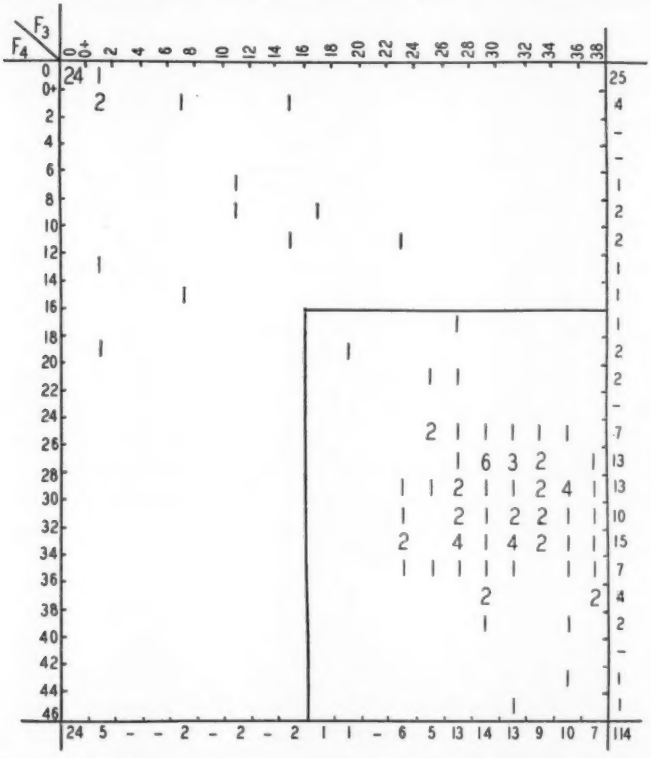

FIGURE 4.—Correlation between the frequencies of wild-type individuals produced in the F_3 and F_4 generations of the same lines. The frequencies are expressed in percent.

high frequency observed in F_3 is probably due to contamination; the F_3 culture contained 149 flies.

The correlation table conveys, first of all, an idea about the validity of the classification of lethals and semilethals. The 24 chromosomes, which behaved in F_3 as carrying lethals, showed a similar behavior in F_4. One of the F_3 semilethals behaved in F_4 as a complete lethal, and another of the F_3 semilethals moved in F_4 into the subnormal range. Two lines which on

the basis of the F_4 data must be classified as semilethals were in the subnormal range in F_5. If the frequencies of the lethals and semilethals were determined on the basis of the F_4, instead of the F_5, data, the figure arrived at would have been 31.3 percent, instead of 30.0 percent. This is a very good agreement.

The strains which gave more than 16 percent wild type in both F_3 and F_4 generations are found in the lower right part of figure 4. If this part is treated as an independent correlation table, a correlation coefficient $r = \pm 0.253 \pm 0.072$ is obtained. This is a weak, but still perceptible, positive correlation. The existence of the genetic viability modifiers is hereby established.

Wild type individuals obtained in F_3 and F_4 of the above experiments are made homozygous for wild third chromosomes. In natural populations, with the possible exception of local colonies *in extremis* reduced to very few individuals, there is no such rigidly enforced homozygosis. The "normal" viability of *D. pseudoobscura* must be looked for in individuals that receive from their parents unlike chromosomes of each kind, or, to put it more exactly, a random sample of the chromosomes present in the population in question. To obtain such a standard of comparison, *Bl Sc* / wild females and males from *different* F_4 cultures were intercrossed. The frequency of the wild type individuals in the resulting offspring is shown in table 3. The

TABLE 3

Viability of types heterozygous for two different third chromosomes extracted from wild populations.

PERCENT OF WILD TYPES	0	...	20–22	22–24	24–26	26–28	28–30	30–32	32–34	34–36	36–38	38–40	40–42	42–44	44–46	46–48	Total
No lethals	—	...	1	3	4	12	18	26	34	37	22	14	6	6	1	1	185
Two lethals	4	...	—	3	3	8	13	22	29	19	10	10	1	3	3	—	124
Total	4	...	1	6	7	20	31	48	63	56	32	24	7	9	4	1	309

distribution curves for the homozygotes and the heterozygotes are shown superimposed on each other in figure 5. It is quite evident that, even disregarding lethals and semilethals, the curve for the heterozygotes is displaced toward the right compared to that for the homozygotes. This is tantamount to saying that the viability of the former is greater than that of the latter.

The task of determining the frequency of the chromosomes containing minor deleterious modifiers is a difficult one, because the intensity of such modifiers ranges from semilethality up to the norm. In working with the Death Valley populations, an attempt was made to estimate this frequency by counting the number of strains which gave less than 34 percent wild

type in both F_3 and F_4. The figure arrived at was 39 per cent of all chromosomes studied, or 43.6 percent of the chromosomes having no lethals or semilethals. An obvious objection against this procedure is that a strain may deviate twice in the minus direction from the expectation without containing a viability modifier, but due to chance alone. On the other hand, strains containing mild deleterious modifiers may, also by chance, give more than 34 percent wild types in one or the other, or in both, generations. This should mitigate the above objection, but nevertheless the figure obtained is at best a rough approximation. In Mexican and Guatemalan populations, strains giving less than 32 percent wild type in both

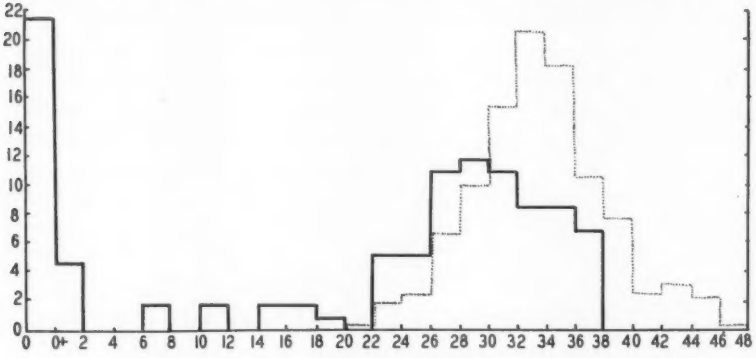

FIGURE 5.—Distributions of the frequencies (in percent) of wild-type individuals produced in cultures where the wild types are homozygous (solid line) and heterozygous (dotted line) for wild third chromosomes.

F_3 and F_4 may be taken to contain deleterious viability genes (the lowering of the standard from 34 percent to 32 percent is due to the average viability of the Mexican and Guatemalan heterozygotes being lower than that of the Death Valley ones, see below). The data presented in figure 4 show that 31 strains gave less than 32 percent, but more than 16 percent, of wild type in F_3 and F_4. This constitutes 27.2 percent of all chromosomes tested and 40.3 percent of those not having lethals or semilethals. The latter figure agrees with that obtained for the Death Valley populations.

More accurate, though less interesting biologically, is the determination of the average reduction of viability produced by homozygosis for a wild third chromosome. Again disregarding the lethals and semilethals, the F_3 cultures have produced on the average 29.84 $\pm$ 0.34 percent (computed from the data in table 2), and the F_4 cultures 30.31 $\pm$ 0.38 percent wild type (computed from figure 4). A comparable figure for the heterozygotes (computed from the data in table 3) is 33.40 $\pm$ 0.18 percent. The difference

is clearly significant. The survival value of homozygotes is distinctly below that of the heterozygotes, so that natural populations resemble to a certain extent the balanced lethal systems used in some laboratory experiments. This conclusion agrees with that arrived at on the basis of the study of Death Valley populations, although the exact figures obtained for the latter are somewhat different, namely 35.52 ± 0.20 percent for heterozygotes and 32.58 ± 0.12 percent for homozygotes. Taken at their face value, the data suggest, therefore, that the genotype of the Mexican and Guatemalan populations is on the whole less favorable than that of the Death Valley ones under the environmental conditions at which the experiments were done. This difference must not be stressed unduly, since the experiments done at different times, actually more than a year apart, are not strictly comparable. Moreover, the mixture of the modifiers contained in the Mexican third chromosomes with those in other chromosomes of the *or pr* and *or Bl Sc pr* strains may be in part responsible.

Lethals and viability modifiers are not the only genetic variants detected in the wild third chromosomes. Although no sharp mutant types, such as are commonly used in linkage studies, were found in the Mexican and Guatemalan materials, at least nine of the homozygotes were phenotypically abnormal in varying degrees. Of these, five were also semilethal, and four possessed normal or subnormal viability. Four were wing characters, two were dwarfish, one had a pale body color, one had short legs and antennae, and one showed black spots on the trochanters of the legs.

Genes modifying the development rate are also common. Table 4 presents the data for eight cultures in which such modifiers are manifestly at work. In Huehuetenango-2, Patzcuaro-2, and Amecameca-19 the early counts give a greater proportion of wild type than do the later ones. Here, consequently, the wild third chromosomes contain genes which speed up the development of the homozygotes (wild-type) relative to that of the heterozygotes (*Bl Sc*). The reverse is observed in the remaining five cultures, in which the early counts show a preponderance of *Bl Sc*, and the late ones an excess of wild-type flies. In these cultures the wild third chromosomes carry modifiers that slow down the development. Following the advice of DR. K. MATHER, the reality of these phenomena was tested by calculating the χ^2 values for heterogeneity, using the BRANDT and SNEDECOR method. The χ^2 values are shown in table 4 together with estimates of P, that is of the probability that such or greater deviations from homogeneity may arise by chance. Except for Orizaba-1, the P values are very small, and hence the heterogeneity is a real one. As expected, in the F_4 generation the strains shown in table 4 have repeated their performance shown by F_3, with the sole exception of Amecameca-19, which in F_4 showed mere traces of an excess of wild-type in the early counts. Since

out of 120 chromosomes tested in F_3, 25 chromosomes contained lethals or extreme semilethals, an opportunity to detect modifiers of the development rate was present in only 95 cultures. Among these, 8 cultures manifested such modifiers, thus giving the frequency 8.4 percent. There is no doubt that this is a gross underestimate compared to the real frequency, because the experiments were so arranged that the detection of the development rate modifiers was made difficult. The parents were allowed to oviposite until their offspring started to hatch, thus obscuring the action not only of the slight but even of the medium strong modifiers.

TABLE 4

Proportions of wild-type and Blade Scute individuals on successive counts in certain F_3 cultures.

DATE	HUEHUE-TENANGO-2		PATZ-CUARO-2		PATZ-CUARO-13		PACHUCA-13		PACHUCA-24		DATE	ORIZABA-1		AMECA-MECA-19		QUEZAL-TENANGO-21	
	WILD	*Bl-Sc*	WILD	*Bl-Sc*	WILD	*Bl-Sc*	WILD	*Bl-Sc*	WILD	*Bl-Sc*		WILD	*Bl-Sc*	WILD	*Bl-Sc*	WILD	*Bl-Sc*
June 21	22	44	20	76	—	10	—	6	—	3	June 19			19	26		
June 24	7	13	1	20	3	38	3	14	—	15	June 21	2	23	5	9	—	15
June 27	3	16	—	45	17	38	7	21	1	51	June 23	1	21	21	36	—	51
June 30	4	8	—	33	18	34	19	37	14	29	June 26	14	45	6	24	2	15
July 4	—	10	—	40	17	18	11	23	15	29	June 29	19	40	4	45	9	13
July 9	—	22	—	39	11	34	2	25	6	30	July 2	11	27	6	36	7	13
July 11					3	11					July 4	12	47	22	68		
											July 9	14	43	5	19		
χ^2	15.7838		42.8639		22.1360		17.4181		27.3419		χ^2	11.3578		22.8096		30.6264	
P	0.01–0.02		<0.01		<0.01		<0.01		<0.01		P	0.10–0.20		<0.01		<0.01	

RECESSIVITY OF THE LETHALS

For an understanding of the population dynamics it is very important to know whether the lethals and other genetic variants present in the wild flies are completely or only partly recessive. The equilibrium frequency of a lethal in a population is determined by the mutation frequency producing that lethal from the normal allele, by the frequency of the reverse mutation, and by selection eliminating the lethal-bearing chromosomes. A completely recessive lethal is sheltered by its normal allele as long as only individuals heterozygous for it are present in the population; the elimination occurs only when homozygotes are produced. If the frequency of a lethal is q, that of the homozygotes is evidently q^2. If, on the other hand, the adaptive value of the heterozygotes is lower than that of the nonlethal homozygotes, the elimination can go on at a much faster rate.

To approach this problem experimentally, intercrosses of *Bl Sc* / wild females and males from *different* F_4 cultures were arranged. Two series of such intercrosses were made: in one of them the flies were taken from cultures known to contain no lethals in the third chromosomes, and in the other both parents carried lethals. The wild-type individuals appearing in

the F_5 of the first series are free of lethals (except where the lethals might have arisen by mutation, which is negligible), and the *Bl Sc* sibs contain only one lethal, namely Blade. In the F_5 of the second series the wild-type flies have two lethals, one in each of their third chromosomes, and the *Bl Sc* sibs have also two lethals, namely Blade and one or the other of the two "natural" ones. It is evident that if the lethals are incompletely recessive, the average frequency of the wild-type individuals in the first series must be greater than that in the second series.

Experiments of the type just outlined were made also with lethal-bearing and with non-lethal bearing chromosomes from the Death Valley populations (DOBZHANSKY and QUEAL 1938b). The results obtained there suggested that at least some of the lethals have slight dominant effects. Since however the two series of the experiments were not run simultaneously, these results were not conclusive, in view of the possible environmental differences between the series. The problem being a rather important one, it was decided to repeat the experiments on a larger scale and with all possible precautions, using Mexican and Guatemalan materials. The new results are shown in table 3. Some of the intercrosses containing lethals gave no wild-type flies at all; cultures of this kind may be disregarded since they obviously represent instances in which the pairs of lethals found in wild populations happened to be alleles. Otherwise, the average frequencies of wild types in the cultures with and without lethals are respectively 33.11 $\pm$ 0.27 percent and 33.59 $\pm$ 0.22 percent. The difference, 0.48 $\pm$ 0.35 percent is less than one and a half time greater than its probable error, and therefore not significant. As an additional check, the average numbers of offspring produced in the cultures of each series were computed, on the assumption that the possible dominant effects of the lethals may cause a drop in the productivity of the flies. The figures obtained were 160.25 $\pm$ 4.12 and 163.11 $\pm$ 3.50 per culture containing and not containing lethals respectively. The result is again negative.

The problem of the recessivity of the naturally occurring lethals is nevertheless not closed. In statistics, negative results are incapable of demonstrating the absence of a supposed effect, although they can show, and in the present case do show, that this effect, if any, is slight. Yet, even a very slight effect of this type is not negligible in population mechanics. An indirect method of attacking the same problem is to compare the elimination rate of the lethals calculated on the assumption that they are completely recessive, with the mutation rate producing lethals. It is hoped to present results obtained with the aid of this method in a separate publication.

DISCUSSION

The genetic structure of Mexican and Guatemalan populations of *D. pseudoobscura* proved to be, in its broad outline, the same as observed pre-

viously in the populations of the Death Valley region. It encompasses an extensive variation in the gene arrangement in the third, contrasting with a relative stability in other chromosomes, some variation in the shape of the Y chromosome, and the presence of an amazing store of recessive lethals, semilethals, deleterious viability modifiers, visible mutants, and modifiers of the development rate. Although it is premature to generalize these results as universal, even for all populations of *D. pseudoobsura*, it is reasonable to assume, as a working hypothesis, that similar conditions may prevail in many sexually reproducing and cross-fertilizing organisms. Pragmatically, differences are however at least as interesting as are the similarities, since the former may permit an insight into the operation of the forces of population mechanics.

The environment in which *D. pseudoobscura* lives below the Tropic in Mexico and Guatemala is obviously distinct from that of the high mountain ranges of the Death Valley region. In Mexican and Guatemalan populations about 30 percent of the third chromosomes carry lethals and semilethals, contrasting with only 15 percent in the Death Valley region. Is there any functional relation between the environmental differences and those in the genetic structure of the fly populations? Three hypotheses, not necessarily alternative to each other, may be advanced in this connection. First, the mutation rate producing lethals may be higher in the Mexican and Guatemalan populations than in the Death Valley ones. Indeed, DEMEREC (1938) and DUBOVSKIJ (1935) found that certain strains of *D. melanogaster* have a greater inherent mutability than others. Since the equilibrium point for a recessive lethal is the square root of the mutation rate, the mutation rate in the Death Valley race must be merely one-fourth of what it is in the Mexican-Guatemalan race. Experiments to test this possibility are in progress. Second, if the lethals are not completely recessive, their frequency may be influenced by selection in the heterozygous state. The stringency, as well as the sign, of the selection may differ in the respective geographic regions. Even if we were able to ascertain that lethals have dominant effects, it would still remain next to impossible to test this hypothesis experimentally. A third possibility, which seems most plausible, is that the greater concentration of lethals in Mexico and Guatemala is due to the effective size of the breeding population being greater in these countries than it is in the Death Valley region.

If there is no reverse mutation from lethal to normal, the equilibrium concentration of a lethal in a population is, as stated above, equal to the square root of the mutation rate from normal to lethal. This is however a limiting case, fully realized only in very large populations, while in smaller ones the equilibrium may be, as shown by WRIGHT (1931, 1937) much lower. In the theoretical example discussed by WRIGHT, the equilibrium

value for a lethal with a mutation rate of 1 : 100,000 is 0.0032 in an effective population of one million or more, and only 0.00008 in a population of ten individuals. It is not possible to tell how great must be the difference between the population sizes to account for a 100 percent increase or a 50 percent reduction in the concentration of lethals, since the relation between these variables in not a linear one. In WRIGHT'S example, a factor of ten can more than double the concentration of lethals in populations of the order from one to ten thousands, and smaller factors suffice for smaller populations. The accumulation of lethals in all parts of the distribution area of *D. pseudoobscura* would have been equal if there were no racial variation in the mutation rate, and if the effective population sizes were large enough throughout to be treated as practically infinite ones. Our data show however that this is not the case.

Although no exact information on the effective population size is available, the following considerations are germane. In exceptionally favorable localities and in favorable season, a hundred or more individuals can assemble in each trap bottle exposed for several hours. Even where the productivity of a trap is very much lower, there is no apparent decrease in the catch if the traps are exposed in the same place day after day. In localities where several species of Drosophila occur together, the compositions of the samples caught in different traps, placed about one or two hundred feet apart, may be very different, proving that the flies entering the traps come from their immediate neighborhoods, and certainly do not travel for miles to reach them. The absolute numbers of the flies present at the peak of the season in mildly favorable forested areas are, it appears, enormous. Yet, to infer from this that the effective sizes of the breeding population must always be practically infinite would be fallacious. Two things must not be lost sight of. First, in a species subject to seasonal fluctuations in numbers, the effective population size is close to that reached at the minimum phase (WIGHT 1938). Second, as shown by DOBZHANSKY and QUEAL (1938a) and by KOLLER (1939), populations inhabiting not only adjacent mountain ranges but even parts of the same mountain range are frequently distinct genetically. The microgeographic variations described in the present article also indicate that the elementary breeding unit may be a population inhabiting a very small territory, although how small that territory may be remains to be determined by future studies.

Curiously enough, the apparent abundance of the flies in Mexico and Guatemala is lower than it is on the mountain ranges of the Death Valley region, although these latter are also by no means the most densely populated places known. The writer collected in Mexico in September, which is the end of the rainy season, and again in March, which is in the second half of the dry period; at both times it proved to be not an easy matter to

assemble adequate samples in many localities. It may also be noted that in the Death Valley region, and in the California mountains in general, *D. pseudoobscura* is by far the commonest species attracted to the traps, while in Mexico and Guatemala it shares the available habitats with a variety of others, among which *D. azteca* and species of *D. hydei* and *D. saltans* groups are equally or more common.

There is however an important difference between the Mexican and Guatemalan populations on one hand and those from the Death Valley region on the other, a difference which has, in fact, provided the stimulus for the present investigation. Beginning with the region lying several degrees of latitude below the Tropic of Cancer, the yearly climatic cycle consists chiefly in an alternation of rainy and dry, rather than of cold and warm, seasons. Despite the fact that *D. pseudoobscura* occurs in the highlands of Mexico and Guatemala and avoids the strictly tropical zone, it can breed throughout the year without major interruptions, and without apparent shrinkages and expansions in numbers. The situation is quite different in the Death Valley region, where the forest belt is so high above sea level that it is covered with snow, or at least subjected to freezing temperatures, for about five months in a year, and in addition is exposed to extreme aridity in late summer. While in Mexico no difference in the apparent density of the flies was observed between September and March, which are climatically very distinct, sharp seasonal fluctuations occur in the California mountains. Although more information is admittedly needed on the seasonal populaton changes than is now available, it is safe to say that the amplitude of the fluctuations in numbers is smaller in *D. pseudoobscura* living below the Tropic than it is in the Temperate Zone. Although the apparent abundance of the flies at the peak of the season is greater in the Death Valley region than it is in Mexico or Guatemala, the abundance at the eclipse stage, and therewith the genetically effective population size, are the opposite. The greater genetic heterogeneity observed in the Mexican and Guatemalan populations is in accord with the expectation arrived at on theoretical grounds.

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SUMMARY

1. Samples of natural populations of *Drosophila pseudoobscura* were collected in eighteen localities in Mexico and Guatemala (figure 3), and a cytogenetic study of the material so obtained was made.

2. Nine distinct gene arrangements in the third chromosome, one of them first described in the present article, occur in Mexico and Guatemala (table 1, figure 1). All but one are phylogenetically interrelated, and belong to the Santa Cruz "family," which is indigenous also in the southwestern United States, but is rare or absent in the Northwest and in British Columbia.

3. The relative frequencies of the gene arrangements vary from locality to locality, showing both the major geographical trends and microgeographic fluctuations.

4. Santa Cruz and Estes Park arrangements, which are sufficiently similar presumably to allow crossing over throughout the length of the chromosome, practically never occur together.

5. Two types of the Y chromosome are found in Mexico, and a third occurs in Guatemala (figure 3).

6. The "sex-ratio" condition is moderately common in Mexico and rare in Guatemala.

7. In Mexico and Guatemala, about 30 percent of the wild third chromosomes contain lethals and semilethals. This contrasts with the 15 percent obtained for the Death Valley region.

8. Among the chromosomes free of lethals and semilethals, a large fraction, perhaps as much as 40 percent, have modifiers deleterious for the viability. Visible mutants and modifiers of the development rate are also common.

9. The adaptive value of individuals homozygous for wild third chromosomes is markedly below that of individuals heterozygous for different chromosomes found in the same population (figure 5). The lethals and other deleterious viability changes carried in wild third chromosomes seem to be completely recessive, although this is not regarded as established beyond doubt.

10. The greater genetic heterogeneity observed in Mexican and Guatemalan populations as compared with those from the Death Valley region is probably due to the effective size of the breeding population being greater in the tropical than in the more northern localities.

LITERATURE CITED

DEMEREC, M., 1937 Frequency of spontaneous mutations in certain stocks of *Drosophila melanogaster*. Genetics **22**: 469–478.

DOBZHANSKY, TH., 1935 The Y chromosome of *Drosophila pseudoobscura*. Genetics **20**: 366–376.
1937 Further data on the variation of the Y chromosome in *Drosophila pseudoobscura*. Genetics **22**: 340–346.

DOBZHANSKY, TH., and QUEAL, M. L., 1938a Genetics of natural populations. I. Chromosome variation in populations of *Drosophila pseudoobscura* inhabiting isolated mountain ranges. Genetics **23**: 239–251.
1938b Genetics of natural populations. II. Genic variation in populations of *Drosophila pseudoobscura* inhabiting isolated mountain ranges. Genetics **23**: 463–484.

DOBZHANSKY, TH., and STURTEVANT, A. H., 1938 Inversions in the chromosomes of *Drosophila pseudoobscura*. Genetics **23**: 28–64.

DUBOVSKIJ, N. V., 1935 On the question of the comparative mutability of stocks of *Drosophila melanogaster* of different origin. C. R. Acad. Sci. U.R.S.S., **4**: 95–97.

KOLLER, P. C., 1939 Genetics of natural populations. III. Gene arrangements in populations of *Drosophila pseudoobscura* from contiguous localities. Genetics **24**: 22–33.

STURTEVANT, A. H., 1937 Autosomal lethals in wild populations of *Drosophila pseudoobscura*. Biol. Bull., **73**: 542–551.

1938 Essays on evolution. III. On the origin of interspecific sterility. Quart. Rev. Biol., **13**: 333–335.

STURTEVANT, A. H., and DOBZHANSKY, TH., 1936 Geographical distribution and cytology of "sex-ratio" in *Drosophila pseudoobscura* and related species. Genetics **21**: 473–490.

WRIGHT, S., 1931 Evolution in mendelian populations. Genetics **16**: 97–159.

1937 The distribution of gene frequencies in populations. Proc. Nat. Acad. Sci., **23**: 307–320.

1938 Size of population and breeding structure in relation to evolution. Science **87**: 430–431.

CROSSING OVER AND HETEROCHROMATIN IN THE X CHROMOSOME OF DROSOPHILA MELANOGASTER*

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INTRODUCTION

IT IS well known that the frequency of crossing over per unit cytological length, either mitotic or salivary, is not constant along the chromosomes of *Drosophila melanogaster*. Furthermore there is ample evidence to show that the centromeric, or spindle attachment, regions of the chromosomes have certain peculiar properties in this respect. Two of the most important of these characteristics are:

(a) Crossing over per unit cytological length near the centromere is lower than elsewhere.

(b) Crossing over is most variable near the centromere, as shown by the effects of temperature changes, age and irradiation.

Now the centromeric regions of the three long chromosomes are also peculiar in another respect; they are largely heterochromatic and "inert," the more distal regions being euchromatic and "active." It is important to know how far the special properties in crossing over of these regions are related to their heterochromatic nature, or on the other hand whether their low and variable crossing over is conditioned by proximity to the centromere. Such information is of value not only in connection with the theory of crossing over but also from the standpoint of the nature of heterochromatin, and of inertness.

This problem has been attacked in the past by OFFERMAN and MULLER (1932) who used two X chromosome inversions, *sc*^{8} and *δ49*, and several X-IV translocations. Their method was to follow crossing over in females homozygous for these structural changes. It was concluded that though the frequency of crossing over of the euchromatic regions was related to their proximity to the centromere, that of the heterochromatin was independent of its position in the chromosome. Mention was made of attempts to follow the variability of crossing over in these changed chromosomes, but no specific results or conclusions are noted.

The many X chromosome inversions now available make it possible to attempt a more extensive analysis of the relations of crossing over, the centromere and heterochromatin. It seems desirable to confine attention to such inversions rather than to consider translocations, as the spatial

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distribution of crossing over in any chromosome is apparently related to the length of the arm in question (MATHER 1936). Certain data of this kind have been obtained by STONE and THOMAS (1935), GRÜNEBERG (1935, 1937) and STURTEVANT and BEADLE (1936), though these authors were largely concerned with other aspects of inversion behavior. The experiments described below are in many ways an elaboration and extension of certain crosses made by these earlier investigators.

I wish to express my gratitude to PROFESSOR T. H. MORGAN for granting me facilities for this work at the California Institute of Technology, while I was holding a Rockefeller International Research Fellowship. I am also indebted to DR. A. H. STURTEVANT and others of the genetical staff of that Institute for their interest and guidance during these investigations. A number of the flies used in the study of the y^4 inversion were kindly recorded for me by DR. P. CH. KOLLER.

EXPERIMENTAL MATERIAL

Crossing over was followed in flies homozygous for X chromosomes having (a) normal sequence, (b) the sc^8 inversion (c) the rst^3 inversion (d) the sc^4 inversion (e) the y^4 inversion. Certain other inversions would have been valuable for the work, but were either not readily available or difficult to use on technical grounds.

The lengths and positions of the inversions are shown, together with the loci used, in figure 1. The locations of the breakage points and gene loci in the euchromatic left section of the chromosome (shown by a solid line) are based on BRIDGES' (1938) salivary map of this element. These loci may be placed on such a map with some precision. The heterochromatic right end of the chromosome (shown as a wavy line) is, however, not portrayed with any attempt at accuracy, other than as to the positions of the breaks relative to each other and to the *bb* locus. Information about the length and structure of this heterochromatin is not good, and in any case the relative length is not the same in salivary and mitotic chromosomes.

Descriptions of the inversions and their phenotypic effects, together with the evidence for the location of their breaks in the places chosen, will be found in the papers cited in the introductory section.

It will be convenient at this point to give the meanings of the gene symbols used in this paper. The inversions are scute-8 (sc^8), scute-4 (sc^4), roughest-3 (rst^3) and yellow-4 (y^4). The name and symbol in each case depends on the mutation or position effect accompanying the inversion. The genes used are yellow (three alleles, *y*, y^2 and y^{31d}), apricot (w^a), echinus (*ec*), crossveinless (*cv*) cut-6 (ct^6), vermilion (*v*), dusky (*dy*), garnet-2 (g^2), forked (*f*), Bar (*B*), carnation (*cr*) and bobbed (two alleles, *bb* and bb^1).

Their positions on the standard map are shown in figure 1 and also given numerically in table 14.

In nearly all cases the crossover data were obtained from single females which were mated with from one to five males in a vial for the first day after emergence. These single females were then allowed to lay in separate bottles for two-day periods. After four such periods, that is, on the ninth day, the surviving females were removed and killed. In the temperature experiments, the females were raised from an early larval stage through pupation and emergence at the chosen temperature. They were further mated and allowed to lay at this same temperature. Thus the data on crossing over at any temperature are from progeny of females whose whole life, after the first few days as larvae, was spent at that tem-

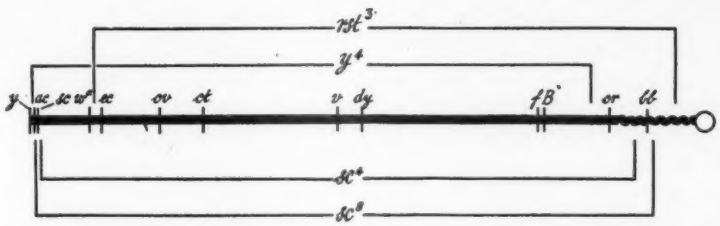

FIGURE 1.—Diagram showing the relative positions of the inversion breaks and of the genes used in these experiments. The solid line indicates euchromatin, and the wavy line heterochromatin. The positions of the various loci in the euchromatin are taken from BRIDGES (1938) map. The loci in the heterochromatin are not accurately placed. Only their relative positions are shown.

perature. The actual progeny of these females, after the removal of the mother, was always raised at 25°C in order to minimize death from overheating.

In all cases it proved possible to obtain adult females at each of the temperatures tried. Sometimes however these females were sterile, or nearly so, at the higher temperatures and so no crossing over data could be obtained. These observations may be of interest and so they are set out in tabular form in table 1. "Partially fertile" indicates that the number of offspring, though considerably reduced, was still appreciable.

TABLE 1

SEQUENCE	19°C	25°C	28°C	30°C.
Normal	Fertile	Fertile	Fertile	Fertile
sc^8	Fertile	Fertile	Fertile	Fertile
rst^3	Fertile	Fertile	Fertile	Partially fertile
y^4	Fertile	Fertile	Partially fertile	Sterile
sc^4	Fertile	Fertile	Almost sterile	Sterile

TABLE 2

Cross $\frac{y^2\ w^a}{cv\ v\ f} \times y^2\ w^a\ cv\ v\ f$ *[Both sexes recorded] Percentages of Recombination.*

REGION	19°C					25°C					28°C				
	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL
y^2–w^a	2.92	0.86	2.30	3.78	2.48	2.47	2.13	2.63	1.95	2.27	3.16	2.46	1.68	1.67	2.15
w^a–cv	17.52	12.97	16.91	14.44	15.22	11.60	13.07	16.72	12.12	13.55	11.65	13.29	17.34	14.83	14.57
cv–v	32.85	25.07	24.84	22.44	24.91	26.91	24.32	17.49	22.90	22.51	23.12	24.38	20.47	18.51	21.33
v–f	24.09	26.80	21.92	25.78	24.56	19.26	20.52	18.73	24.10	20.61	19.97	22.19	23.94	23.63	22.07
Number of flies	137	347	479	450	1413	405	658	646	668	2377	601	730	894	897	3122

χ^2 *analysis*	Degrees of Freedom	y^2–w^a		w^a–cv		cv–v		v–f	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P
Age variation	9	13.086	0.20–0.10	21.963	<0.01	31.071	<0.01	15.075	0.10–0.05
Temperature variation	2	0.487	0.90–0.80	2.237	0.50–0.30	7.156	0.10–0.05	6.115	0.10–0.05

Note to Tables 2–13. The values in the body of the table are all recombination percentages except for the bottom row which shows the numbers of flies on which the recombinations are based. The "Total" columns give the percentages, and numbers of flies, for the temperature in question when the flies are summed regardless of age. Thus there are 1413 flies in all at 19°C in table 2. This is the sum of the four age items, 137, 347, 479 and 450.

The results are shown in tables 2–13 in the form of recombination percentages together with the number of flies counted. Details of segregations cannot be given as they would occupy too much space.

The statistical analyses were concerned with the frequencies of recombination, each marked section of the chromosome being treated individually. In all cases the χ^2 test was used, χ^2 being calculated either by the method appropriate to a 2×2 contingency table or from BRANDT and SNEDECOR'S formula (MATHER 1938). The χ^2's were analyzed into two parts, one concerned with variation in recombination following temperature changes and the other relating to age variation. Table 2, for example, contains the results of an experiment involving three temperatures with four ages at each temperature. Thus there are 12 groups, and hence 11 degrees of freedom. Now if we sum all the data for any one temperature regardless of age (given as Totals in table 2) we have three groups separated solely on the basis of temperature. A test of this "temperature variation" may be made by calculating a χ^2 for two degrees of freedom. Within each temperature are four age groups, which may be compared by calculating a χ^2 for three degrees of freedom. We may however sum the three age χ^2's, one from each temperature, and obtain a general "age variation" χ^2 for 9 degrees of freedom. Thus the 11 degrees of freedom between the 12 recombination determinations are divided into two for temperature and nine for age variation. Below each table are given the χ^2 analyses and the appropriate probabilities as determined by reference to a table of χ^2 such as that given by MATHER (1938).

OBSERVATIONS

(a) *Control data.* Three different crosses made using the normal chromosome sequence were

1. $$\frac{y^2\ w^a}{cv\ v\ f} \times y^2\ w^a\ cv\ v\ f$$

2. $$\frac{v\ B \quad bb}{cr} \times v\ cr\ bb^1$$

3. $$\frac{v\ f}{bb} \times v\ f\ bb^1$$

In both (2) and (3) other genes were segregating but were disregarded for various reasons. There is no reason to believe that any of these extraneous segregations would upset the results from the genes followed.

The cross (1) was made three times. On each occasion, if more than one

TABLE 3

Cross $\frac{y^2\ w^a}{cv\ v\ f} \times y^2\ w^a\ cv\ v\ f$ *[Both sexes recorded] Percentages of Recombination.*

REGION	19°C					25°C					28°C				
	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL
y^2–w^a	0.89	2.60	2.85	2.67	2.17	1.12	2.19	1.61	0.54	1.45	1.61	2.80	1.01	1.27	1.75
w^a–cv	14.73	17.65	19.01	18.32	17.26	13.93	14.18	16.50	13.41	14.72	16.60	15.21	12.79	13.92	14.55
cv–v	26.04	24.22	27.19	25.76	25.78	25.39	28.07	25.81	20.83	25.25	24.54	24.47	23.23	23.33	23.92
v–f	27.98	26.64	25.86	28.63	27.30	27.42	24.42	25.06	21.56	24.53	25.16	24.13	23.91	22.97	24.11
Number of flies	672	578	526	524	**2300**	445	684	806	552	**2487**	807	1177	1188	553	**3725**

χ^2 *analysis*	Degrees of Freedom	y^2–w^a		w^a–cv		cv–v		v–f	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P
Age variation	9	25.986	<0.01	12.121	0.30–0.20	10.803	0.30–0.20	6.968	0.70–0.50
Temperature variation	2	3.651	0.20–0.10	9.072	0.02–0.01	3.009	0.30–0.20	8.331	0.02–0.01

temperature was used, the heterozygous females used were all sisters, though raised at different temperatures. Hence their results are strictly comparable, if it is granted that age of mother or father has no effect on the frequency of crossing over in the daughter. Both male and female progeny were recorded in each case, with the exception of the 30°C cross, where only males were used. In this case the males used in the cross were of a slightly different constitution (table 4). Their sisters were of type 3.

The heterozygous females used at the different temperatures in crosses 2 and 3 were also progeny of the same parents.

TABLE 4

Cross $\frac{y^2\ w^a \qquad bb}{cv\ v\ f} \times y^2\ cv\ v\ f\ cr\ bb^1$ *[Males recorded] Percentages of Recombination.*

REGION	30°C				
	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL
y²–w^a	3.24	1.52	2.06	3.92	3.26
w^a–cv	18.77	16.46	15.10	18.14	16.92
cv–v	24.92	25.57	26.78	26.96	25.97
v–f	32.68	29.62	5.36	29.41	29.15
Number of flies	309	395	351	204	**1295**

The control data are summarized in tables 2–6. Results for different temperatures where shown in the same table, were obtained from sister flies, as noted above. Where on different tables the mother flies were not sisters. In general the results are much as expected, though recombination throughout is higher than would be expected from the standard map. The data confirm STERN'S (1926) conclusions in showing that the region *f-bb* shows temperature variations in recombination, and even go further in narrowing down this effect to the *cr-bb* region. The other regions also show significant age variations, particularly in the data of table 3, but as these are not consistent from one group of data to the next it seems safe to suppose that these are not due to genuine age and temperature effects. Their cause is not obvious.

The data of table 4 are particularly aberrant in showing very high recombinations for all the regions. These results are, however, not strictly comparable with any of the others, except for the *v-f* region. For this region they may be compared with their sisters recorded in table 6. This comparison shows the figures of table 4 to be unduly high; they should therefore be treated with considerable caution. In general the age and temperature effects occur consistently only in the *f-bb* region.

One technical point may properly be considered here. In the crosses of

TABLE 5

Cross $\frac{v\ B}{cr} \times \frac{bb}{}$ *v car bb*^{1} *[Females recorded] Percentages of Recombination.*

REGION	25°C					28°C					30°C
	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL	TOTAL
v–B	23.93	25.18	25.50	27.32	25.36	26.43	22.28	32.00	22.73	26.08	21.13
B–cr	11.11	7.29	6.00	6.01	7.40	10.13	10.39	8.89	8.33	9.54	8.25
cr–bb	4.27	2.82	3.50	3.28	3.38	7.04	5.45	6.22	6.81	6.36	4.64
Number of flies	234	425	400	183	1242	227	202	225	132	786	194

χ^2 *analysis*	Degrees of Freedom	*v–B*		*B–cr*		*cr–bb*	
		χ^2	P	χ^2	P	χ^2	P
Age variation	6	12.436	0.05–0.02	6.962	0.30–0.20	1.613	0.98–0.95
Temperature variation	2	2.037	0.50–0.30	2.902	0.30–0.20	9.853	<0.01

tables 5 and 6, segregation for the extreme bobbed character was observed and as was expected, the extreme bobbed females failed to equal their not bobbed sisters in number. Now MORGAN, BRIDGES and SCHULTZ (1935) advocate treating the *bb* locus as a lethal and discarding all the extreme bobbed flies. Equal numbers of flies are then raised from crosses having *bb* in coupling and in repulsion with the other genes, in order to balance the effect. I have not followed this practice as there is internal evidence which shows that the bobbed flies may be used. The extreme bobbed flies will not give different recombination values for the various regions unless their mortality is affected differentially by the other genes.

This question may be approached statistically. If we make a 2×2 table having recombination and non-recombination for the region in question along one margin and the numbers of *bb* and not-*bb* flies along the other, we may calculate a χ^2 which will detect differences in recombination between *bb* and not-*bb* flies. They should, of course, give the same recombination value. The χ^2 values, calculated separately for each age group at each temperature, wherever the numbers are sufficiently large, may then be summed for each region. In this way we obtain a χ^2 testing the agreement, over all ages and temperatures, of the *bb* and not-*bb* flies in the recombination shown for either the *v-f* region or the *f-bb* region or any other region followed. Each 2×2 table in the analysis will contribute one degree of freedom (MATHER 1938). The results of this analysis are:

REGION	DATA FROM CROSS GIVEN IN TABLE	χ^2	DEGREES OF FREEDOM	PROBABILITY
v–f	6	15.4941	11	0.20–0.10
f–bb	6	14.8671	11	0.20–0.10
v–B	5	14.9245	11	0.20–0.10
B–cr	5	5.7476	9	0.80–0.70
cr–bb	5	9.4679	9	0.50–0.30

It will be observed that in no case is the χ^2 significant. Hence for the purpose of determining the recombination values, we may add the *bb* flies to the not-*bb* flies, and need not discard them as has been advocated. Further results illustrating the same point will be given in the section dealing with the *rst*^{3} crosses.

(b) *Inversion sc*^{8}. This inversion was used quite extensively. All the genes employed in the experiments were introduced by double crossing over, with the exception of the yellow markers. Two ways of achieving this were used. In the first place the *sc*^{8} deficiency chromosome was employed. This is *sc*^{8} with the uninverted left section and a small amount

TABLE 6

Cross $\frac{v\ f}{bb} \times v\ f\ bb^1$ *[Females recorded] Percentages of Recombination.*

REGION	25°C				28°C					30°C				
	2–3 DAYS	4–5 DAYS	6–7 DAYS	TOTAL	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL
v–f	22.49	20.93	24.12	22.38	21.56	27.00	24.75	22.01	24.32	24.31	24.11	23.48	23.94	23.95
f–bb	12.43	16.28	9.41	13.00	16.51	15.26	16.67	17.15	16.31	17.65	16.50	21.95	15.96	18.20
Number of flies	169	215	170	554	218	426	408	309	1361	255	394	328	188	1165

χ^2 *analysis*	Degrees of Freedom	*v–f*		*f–bb*	
		χ^2	P	χ^2	P
Age variation	8	4.101	0.90–0.80	9.135	0.50–0.30
Temperature variation	2	0.829	0.70–0.50	7.421	0.05–0.02

of the inverted heterochromatin missing. The deficiency covers yellow and may be used to mark that locus. The second marker was the mutant y^{31d}, introduced into the sc^8 chromosome by X-irradiation. The sc^8 deficiency chromosome requires further comment. The deficiency for the inverted heterochromatin was detected by showing that it was deficient for *bb*. This was done by crossing to *bb* and bb^1 stocks. With *bb* it gives extreme bobbed females and with bb^1 it is lethal. Secondly this chromosome shows a very peculiar type of segregation from normal sc^8 chromosomes. In the daughters of a sc^8/sc^8 def. female the deficiency is recovered just twice as frequently as the corresponding portion of the other chromosomes. The segregation for other loci in such flies depends on the closeness of their linkage to the yellow locus. The reasons for this behavior are not fully known, but it seems to be bound up with a maternal effect. However, the recombination values shown by such females agree reasonably well with those shown by sc^8/sc^8 flies and so may be used (tables 7–9).

The sc^8 recombination values are given in tables 7–9. In each case the females used at different temperatures, where shown in the same table, were from the same parents, as in the case of the control data.

It is unfortunately true that in these crosses, as in the controls, a certain amount of inconsistent variation is shown by nearly all the regions followed. The only region to show consistent variability in recombination with temperature is however the *y-f* region. There is a suggestion of variability with temperature in the *cv-w*^{a} section but it is not particularly clear and cannot be taken with any emphasis. The variability of the *y-f* section with temperature is further checked by the rst^3 data given in the next section.

In table 10 are the results of a more complex determination of crossing over in the sc^8 chromosome. This was done solely at 25°C, and no account was taken of age of mother. Eight loci were followed and this allowed a rather detailed analysis of the distribution of crossing over in this chromosome to be made. An attempt was made to follow the sable (*s*) gene in addition; but for a number of reasons, notably the difficulty of classification of *s* on y^{31d}, complicated by the apparent segregation of some autosomal modifiers of *s*, the attempt was given up. Sable did not segregate visibly in the daughters as the recessive fathers were not carrying this gene. The bb^1 of the test males had no effect as the mothers were homozygous not-*bb*.

(c) *Inversion* rst^3. Only one type of cross was used with this inversion. This was

$$\frac{rst^3 \quad y\ bb\ cr}{rst^3 \quad bb\ \ f\ v\ cv} \times y^2\ cv\ v\ f\ cr\ bb^1$$

TABLE 7

Cross $\frac{sc^8\ y^{31d}\qquad w^a}{sc^8\qquad f\ v\ cv} \times y^2\ w^a\ cv\ v\ f$ *[Both sexes recorded] Percentages of Recombination.*

REGION	19°C					25°C					28°C				
	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL
y^{31d}–*f*	16.97	17.33	14.79	15.77	16.22	16.77	14.79	13.41	14.59	14.76	17.36	18.18	17.31	18.44	17.81
f–*v*	26.79	24.65	26.04	26.34	25.94	23.34	22.33	23.51	24.17	23.38	23.78	25.13	20.81	22.46	23.05
v–*cv*	20.25	20.39	19.63	19.05	19.82	17.66	17.73	19.45	17.98	18.24	18.67	20.86	18.03	17.44	18.86
cv–w^a	9.00	8.34	7.30	8.66	8.33	9.15	5.88	5.76	7.97	7.09	7.85	9.80	8.97	9.03	8.98
Number of flies	978	1079	1014	1097	**4168**	787	1021	1059	1179	**4046**	841	1122	1115	797	**3875**

χ^2 *analysis*	Degrees of Freedom	y^{31d}–*f*		*f*–*v*		*v*–*cv*		*cv*–w^a	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P
Age variation	9	13.491	0.20–0.10	8.784	0.50–0.30	6.710	0.70–0.50	14.878	0.10–0.05
Temperature variation	2	13.550	<0.01	11.142	<0.01	3.375	0.20–0.10	10.998	<0.01

It was believed at first that only the *rst*^{3} *y cr* chromosome was carrying *bb* and it was planned that this locus should be followed. The results showed unequivocally, however, that both chromosomes carried *bb*. Since all the daughters were of the extreme bobbed type, this locus could not be used. In most cases only the sons of the cross were recorded as, at that time, suspicion of the effects of extreme bobbed on recombination determination was felt. In some cases, however, it was necessary to use some of the female progeny in addition to their brothers, owing to the low number of the latter. These cases are confined to the 25°C data. In these cases, as in the corresponding cases of the control data, the validity of the grouping of males and females was tested. (In the control series the flies were all of one sex but differed in their phenotype for *bb*.) A 2×11 table was prepared. The classification one way was into eleven classes, being the complement-

TABLE 8

Cross $\frac{sc^8\ y^{31d}}{sc^8}\ \frac{w^a}{f\ v\ cv} \times y^2\ w^a\ cv\ v\ f$ *[Both sexes recorded] Percentages of Recombination.*

REGION	30°C				
	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL
y^{31d}–*f*	19.66	15.84	17.09	17.63	17.58
f–*v*	21.88	19.57	18.18	21.58	20.33
v–*cv*	19.32	16.55	14.73	17.81	17.13
cv–w^a	7.35	5.69	6.18	9.17	7.10
Number of flies	585	562	550	556	2253

ary non-crossover classes, the complementary single crossover classes for each of the four regions, *y*–*cr*, *cr*–*f*, *f*–*v* and *v*–*cv*, and lastly the summed double crossovers. These were summed, as the individual double crossover classes contained but few individuals. The classification the other way was into extreme *bb* females and normal males. Using BRANDT and SNEDECOR'S method to a compute a χ^2, having 10 degrees of freedom, for agreement between the two sexes in the frequencies with which they showed flies of these eleven classes, it was found that two such tables gave the following results:

DEGREES OF FREEDOM	FIRST TABLE FOR 2–3 DAYS AT 25°C		SECOND TABLE FOR 8–9 DAYS AT 25°C	
	χ^2	PROBABILITY	χ^2	PROBABILITY
10	15.778	0.20–0.10	5.879	0.90–0.80

It is thus clear that the sexes are giving no evidence of differences in the recombination frequencies of the four regions and many be added for the

TABLE 9

Cross $\frac{sc^8\ def}{sc^8} \times \frac{u^a}{f\ v\ cv}$ $y^2\ w^a\ cv\ v\ f$ *[Both sexes recorded] Percentages of Recombination.*

REGION	19°C					25°C				
	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL
def–f	13.83	9.45	14.02	11.72	12.08	11.54	16.45	18.54	15.91	16.29
f–v	13.83	22.87	20.79	26.16	22.43	19.23	22.68	21.72	20.65	21.40
v–cv	25.53	19.51	14.95	21.53	18.98	17.52	13.61	17.98	17.85	16.57
cv–w^a	3.19	3.66	7.24	5.18	5.34	1.71	4.16	3.18	5.59	3.92
Number of flies	94	328	428	367	1217	234	529	534	465	1762

χ^2 *analysis*	Degrees of Freedom	*def–f*		*f–v*		*v–cv*		*cv–w*^{a}	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P
Age variation	6	9.877	0.20–0.10	8.986	0.20–0.10	13.564	0.05–0.02	13.119	0.05–0.02
Temperature variation	1	10.248	<0.01	0.453	0.70–0.50	2.889	0.10–0.05	3.406	0.10–0.05

present purpose. This was then done and no further distinction was drawn between the normal males and the extreme bobbed females. This agrees with the absence of effect of extreme *bb* on apparent recombination in certain regions of the normal chromosome.

The *rst*^{3} data are given in table 11. They are quite unambiguous in showing a temperature variation in the frequency of recombination in the $y-cr$ region but in no other section of the chromosome. This is in general better shown here than by the *sc*^{8} results. Certainly the data from both inversions taken together bring out the variability of the $y-f$ or $y-cr$ region very well.

TABLE 10

$$Cross\ \frac{sc^8\ y^{31d}\quad f\quad dy\quad cv\quad w^a}{sc^8\quad cr\quad s\quad ct^6\quad ec} \times y^2\ w^a\ ec\ cv\ ct^6\ dy\ f\ cr\ bb^1\ [Females\ recorded].$$

REGION	y^{31d}–cr	cv–f	f–dy	dy–ct^6	ct^6–cv	cv–ec	ec–w^a	NUMBER OF FLIES
RECOMBINATION	4.99	10.81	22.47	14.89	3.86	3.02	0.98	1424

The other striking feature of the *rst*^{3} results is the large recombination value of the $y-cr$ region (see also GRÜNEBERG 1935, 1937). This will be discussed later.

(d) *Inversion sc*^{4}. The cross

$$\frac{sc^4\ y\qquad\qquad w^a}{sc^4\ y\ \ g^2\ ct^6\ ec} \times B$$

was used, only the males being recorded (table 12). It proved impossible to obtain many flies at 28°C, so no attempt to follow the variability of the recombination values was made. The results are of some interest in connection with the so-called spindle fibre effect.

(e) *Inversion y*^{4}. A number of flies were raised from the cross

$$\frac{y^4}{y^4\ f\ v\ cv} \times y^2\ cv\ v\ f$$

at 25° and 28°C. The results are given in table 13. Little need be said about them. There is no evidence of age or temperature variation in either region. This is not unexpected, in view of the length and position of the inversion.

DISCUSSION

The "spindle fibre effect"

Table 14 shows a summary of the frequencies of recombination observed in the different regions at 25°C. The standard map differences are also

TABLE 11

Cross $\frac{rst^2 \;\; y \;\; bb \;\; cr}{rst^2 \;\; bb \;\; f \;\; v \;\; cv} \times y^2 \; cv \; v \; f \; cr \; bb^1$ *[Only males recorded in majority of cases] Percentages of Recombination.*

REGION	25°C					28°C					30°C
	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL	TOTAL
y–cr	16.79	16.47	12.42	16.20	15.56	19.67	19.25	18.33	20.61	19.40	23.53
cr–f	9.16	7.17	9.59	9.20	8.75	12.20	10.28	9.64	9.90	10.49	9.09
f–v	26.15	25.19	26.80	26.00	26.01	27.50	26.73	25.56	27.87	26.40	21.39
v–cv	20.04	23.45	18.95	21.80	21.11	18.76	18.69	17.20	22.00	18.67	25.67
Number of flies	524	516	459	500	**1999**	549	535	622	459	**2201**	**187**

χ^2 *analysis*	Degrees of Freedom	*y–cr*		*cr–f*		*f–v*		*v–cv*	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P
Age variation	6	5.489	0.50–0.30	4.647	0.70–0.50	14.367	0.05–0.02	15.990	0.02–0.01
Temperature variation	2	14.898	<0.01	3.713	0.20–0.10	2.245	0.70–0.50	7.675	0.10–0.05

given for comparison. It will be seen that the normal sequence results agree reasonably well with the standard, though they are in general slightly higher. The proximal regions are slightly more variable than the rest, as might be expected from the results of GOWEN (1918).

On turning to the results from the sc^8 chromosome we find that the region w^a-ec, normally distal, on being placed close to the centromere becomes genetically much shorter. It shows about a quarter of its normal frequency of crossing over. This is a clear manifestation of the so-called spindle fibre effect. The effect decreases in potency as we proceed distally along the chromosome. The region $ec-cv$ is about 37 percent of normal, the $cv-ct$ region 61 percent of normal and the rest almost unchanged, except for $f-cr$, which is now nearly twice as long as usual. In this case it is the reverse of the spindle fibre effect, observed when a normally proximal region is displaced distally. Are these effects on crossing over genuinely caused by the centromere or are they the effect of heterochromatin? The

TABLE 12

Cross $\frac{sc^4\ y}{sc^4\ y}\ \frac{\qquad w^a}{g^2\ ct^6\ ec}\times B$ *[Males recorded] Percentages of Recombination.*

REGION	g^2–ct^6	ct^6–ec	ec–w^a	NUMBER OF FLIES
TOTAL	24.38	12.60	2.19	365

answer is obtained by comparison with the sc^4 results. In the latter, the w^a-ec region is about 50 percent normal, that is, it is showing a smaller spindle fibre effect than in sc^8. Now it is known that the right break of sc^4 is much distal to the right break of sc^8 (GERSHENSON 1935). In the latter, w^a-ec is nearer to the centromere but adjacent to a smaller section of heterochromatin than in sc^4. Now the greater reduction in sc^8 as compared with sc^4 is easily explained as due to a stronger centric effect, following greater proximity to the centromere. On the other hand if the spindle fibre effect is supposed to be due to the heterochromatin, this result is difficult to understand, as it would mean that the smaller portion of heterochromatin is having a greater effect than the larger one while the $f-cr$ region at the other end is showing the reverse, in that $f-cr$ is genetically longer in sc^8 than in normal sequence. Thus the supposition that the spindle fibre effect is a genuine centric action seems more reasonable. The behavior of the $ec-ct^6$ region is in agreement with this view.

The rst^3 data support those from sc^8 in showing that proximal regions displaced distally have increased crossing over, $f-cr$ being here 8.8. This value probably does not differ in reality from the 10.8 of sc^8.

The y^4 results are of negative interest in helping to demonstrate the

absence of any effect when the right break of the inversion is not close to the centromere.

Crossing over in the heterochromatin

If the low crossing over per unit cytological length in the proximal euchromatin is due to an effect of the centromere and not to some property of the adjacent heterochromatin, the question immediately arises as to whether the heterochromatin itself fails to show crossovers because it is near to the centromere or whether it is by nature incapable of free crossing over. The behavior of the sc^8 and rst^3 chromosomes supplies information on this point.

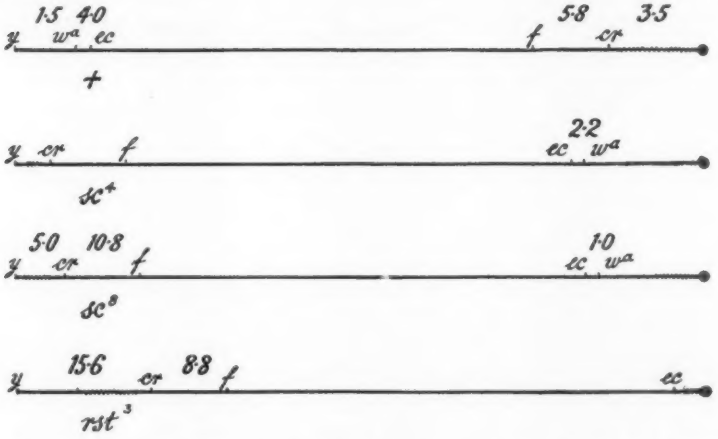

FIGURE 2.—Diagram to illustrate the "spindle fibre" effect. The solid line indicates euchromatin and the wavy line heterochromatin. The numbers above the symbols are the recombination percentages observed.

In the case of sc^8 the left break of the inversion is between achaete and scute (STURTEVANT and BEADLE 1936) and so *y* serves as a good marker of this point. If crossing over between *y* and *cr* is followed in the sc^8 chromosome, a comparison with the value of 3.5 between *cr* and *bb*, which genetically speaking practically coincides with the centromere, yields information of this type. The value observed in experiment was 5.0 percent (table 10) which shows a very low excess over the standard 3.5 percent. Such an excess could be accounted for by the increase of crossing over observed in distally displaced proximal regions, as in the case of *f*—*cr*. The situation is somewhat different in the case of rst^3. Here the left break lies between w^a and *ec*, probably nearer the former. Thus there is a section of euchromatin undisturbed near yellow. Its length is at most 5.0 crossover units and probably much less. At 25°C, 15.6 percent re-

TABLE 13

Cross $\frac{y^4}{y^4\ f\ v\ cv} \times y^2\ cv\ v\ f$ *[Both sexes recorded] Percentages of Recombination.*

REGION	25°C					28°C				
	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL	2–3 DAYS	4–5 DAYS	6–7 DAYS	8–9 DAYS	TOTAL
f–v	26.50	24.20	26.35	25.66	25.72	25.32	29.71	26.88	30.40	27.97
v–cv	20.30	20.17	21.78	21.68	21.09	18.83	16.57	19.35	16.00	17.81
Number of flies	532	595	831	678	**2636**	154	175	186	125	**640**

χ^2 *analysis*	Degrees of Freedom	*f–v*		*v–cv*	
		χ^2	P	χ^2	P
Age variation	6	3.719	0.80–0.70	1.759	0.95–0.90
Temperature variation	1	0.846	0.50–0.30	3.406	0.10–0.05

TABLE 14

In each case the figures between the symbols, on the line marked Standard, give the standard map distances between those genes in the normal sequence. The figures below indicate the recombination percentages observed in the flies recorded in the table denoted at the left, at 25°C.

Normal	Standard	*y* 1.5 w^a 4.0 *ec* 8.2 *cv* 6.3 ct^6 13.0 *v* 3.2 *dy* 20.5 *f* 0.3 *B* 5.5 *cr* 3.5 *bb*
	Table 2	←2.3→←13.6→←22.5→←20.6→
	Table 3	←1.5→←14.7→←25.3→←24.5→
	Table 5	←22.4→←13.0→
	Table 6	←25.4→←7.4→←3.4→
sc^8	Standard	*y*—*bb* 3.5 *cr* 5.8 *f* 20.5 *dy* 3.2 *v* 13.0 ct^6 6.3 *cv* 8.2 *ec* 4.0 w^a
	Table 7	←14.8→←23.4→←18.2→←7.1→
	Table 9	←16.3→←21.4→←16.6→←3.9→
	Table 10	←5.0→←10.8→←22.5→←14.9→←3.9→←3.0→←1.0→
rst^3	Standard	*y*—*bb* 3.5 *cr* 5.8 *f* 23.7 *v* 19.3 *cv*
	Table 11	←15.6→←8.8→←26.0→←21.1→
sc^4	Standard	g^2 24.4 ct^6 14.5 *ec* 4.0 w^a
	Table 12	←24.4→←12.6→←2.2→
y^4z	Standard	*f* 23.7 *v* 19.3 *cv*
	Table 13	←25.7→←21.1→

combination was observed between *y* and *cr* in homozygous *rst*^{3} females (GRÜENBERG, 1937, gave even larger values). Allowing 5 percent for the included remnant of euchromatin, this leaves nearly 11 units for crossing over between *cr* and the breakage point. It would then seem almost beyond doubt that, by comparison with the 3.5 of the normal and the 5.0 of *sc*^{8}, some crossing over must be taking place in the distal heterochromatin of *rst*^{3}. The cause of the discrepancy between *sc*^{8} and *rst*^{3} is not clear, as *sc*^{8} certainly includes, according to GERSHENSON, a considerable piece of inverted heterochromatin. It would be of great interest to obtain a crossover between *rst*^{3} and *sc*^{8} or *rst*^{3} and *sc*^{4} to see how much more heterochromatin is inverted in *rst*^{3} than in *sc*^{8}. It is doubtful if the excess is sufficient to account for the difference in the observed recombination values.

Another possibility exists. If there is a small distal region of low crossing over near yellow, the heterochromatin of the *sc*^{8} inversion might lie wholly or largely in this area and show little if any more crossing over than in its normal place; whereas in *rst*^{3} the remaining euchromatin between *y* and the inversion would keep the inverted heterochromatin in the region of high crossing over. This combined with some excess of inverted heterochromatin in *rst*^{3} as compared with *sc*^{8} might perhaps account for the difference.

Such a distal region of low crossing over, which CHARLES (1938) has supposed to be equal to the centric region, must in fact be shorter than the latter. The evidence from *sc*^{8} shows this well. The region *f*—*cr* is clearly nearer to *y* in *sc*^{8} than it is to the centromere in the normal sequence because the inversion, while almost involving *y*, does not involve all the heterochromatin. Yet this region shows more crossing over in *sc*^{8} than in the normal chromosome.

Whatever the situation with regard to the distal region of low crossing over and the difference between *rst*^{3} and *sc*^{8}, there can be little doubt that in *rst*^{3} the heterochromatin is showing some crossing over, though whether heterochromatin can cross over so freely as euchromatin is an unanswered question. It then follows that the frequency of crossing over in heterochromatin, as in euchromatin, is dependent on the distance from the centromere.

The effect of temperature on crossing over

We have seen above that most probably the "spindle fibre" effect is governed by the centromere and that heterochromatin can most likely cross over when displaced from its position near the centromere to one where crossing over is more favoured. The heterochromatin is thus similar to the euchromatin in respect of some of its behavior in crossing over.

We must now turn to the question of the cause of the high temperature

sensitivity of crossing over near the centromere in the normal chromosome. Here the answer is different.

The data of tables 2–6 confirm STERN's (1926) conclusion that temperature affects the frequency of crossing over in the $f-bb$ region and nowhere else, in the normal chromosome. The data of table 6 further narrow down the seat of the main disturbance to the $cr-bb$ section. This could clearly be either another effect of the centromere or a peculiar property of the heterochromatin, which may also be expressed by adjacent euchromatin.

Turning to the sc^8 and rst^3 data once more we possibly find slight evidence of variability with temperature of crossing over in the now proximal w^a-cv region in sc^8. On the other hand the distal $y-f$ region of sc^8 and $y-cr$ region of rst^3 show a marked and consistent variability of crossing over with temperature changes. The intermediate regions show some spasmodic variability which is unrelated to temperature.

Thus we here have clear evidence of a property peculiar to heterochromatin. Whether proximally or distally placed, its frequency of undergoing crossing over is very much more affected by environmental conditions than is the case with the euchromatin. There is no real evidence of any relation between variability and distance from the centromere, as the possible sensitivity of w^a-cv in sc^8 may be ascribed to its being in juxtaposition to the inverted heterochromatin. (It may be noted that the results of GRAUBARD (1932) on temperature effects in chromosome II fall into line if we suppose his inversion included no heterochromatin.)

Thus of the two peculiarities with regard to crossing over of the centric region of the normal chromosome, one, the low frequency, is an effect of the centromere while the other, the sensitivity to environmental changes, is a property of the heterochromatin. Further, though the heterochromatin most probably resembles the euchromatin in showing a similar relation in crossing over to position with respect to the centromere, it is distinct from the euchromatin in its susceptibility to environmental conditions.

SUMMARY

Crossing over was followed in females homozygous for the normal sequence and four inversion sequences in the X chromosome, different temperatures being used.

The results indicate that:

(a) crossing over in the euchromatin is dependent upon the distance from the centromere.

(b) the heterochromatin can show crossing over, the frequency obtained being dependent on distance from the centromere.

(c) high sensitivity of crossing over to temperature is a property of the heterochromatin.

Thus of the two special properties of the centric region in crossing over, that of the low frequency is a property of adjacency to the centromere, while that of variable frequency is a property solely of the heterochromatin.

LITERATURE CITED

BRIDGES, C. B., 1938 A revised map of the salivary gland X chromosome of *Drosophila melanogaster*. J. Hered. **29**: 11–13.

CHARLES, D. R., 1938 The spatial distribution of crossovers in X chromosome tetrads of *Drosophila melanogaster*. J. Genet. **36**: 103–26.

GERSHENSON, S., 1933 Studies on the genetically inert region of the X chromosome of Drosophila, I. Behavior of an X chromosome deficient for a part of its inert region. J. Genet. **28**: 297–314.

GOWEN, J. W., 1919 A biometrical study of crossing over. Genetics **4**: 205–50.

GRAUBARD, M., 1932 Inversion in *Drosophila melanogaster*. Genetics **17**: 81–106.

GRÜNEBERG, H., 1935 A new inversion of the X chromosome in *Drosophila melanogaster*. J. Genet. **31**: 163–84.

1937 The position effect proved by a spontaneous reinversion of the X chromosome of *Drosophila melanogaster*. J. Genet. **34**: 69–90.

MATHER, K., 1936 The determination of position in crossing over. I. *Drosophila melanogaster*. J. Genet. **33**: 207–35.

1938 The measurement of linkage in heredity. pp. 132. London, Methuen.

MORGAN, T. H., BRIDGES, C. B., and SCHULTZ, J., 1935 Report of investigation on the constitution of the germinal material in relation to heredity. Carneg. Inst. Wash. Year Book **34**: 284–81.

OFFERMAN, C. A., and MULLER, H. J., 1932 Regional differences in crossing over as a function of the chromosome structure. Proc. 6th Int. Cong. Genet. **2**: 143–5.

STERN, C., 1926 The effect of temperature and age on crossing over in the first chromosome of *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **12**: 530–2.

STONE, W., and THOMAS, I., 1935 Crossover and disjunctional properties of X chromosome inversions in *Drosophila melanogaster*. Genetica **17**: 170–84.

STURTEVANT, A. H., and BEADLE, G. W., 1936 The relation of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21**: 554–604.

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